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Final Report

Comprehensive Clinical Phenotyping & Genetic Mapping for the Discovery of Autism Susceptibility Genes

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14. ABSTRACT

Autism is the fastest growing developmental disability in the US, costing more than $90 billion/yr, and is, therefore, extremely important to the DoD. According to the Military Times (Robert Dorr, 8/13/11), "autism is the most prevalent special-needs issue in military families." Autism spectrum disorders (ASDs) have a frequency of ~1/88 in the general population (1) and an estimated frequency of 1/88 in the military (Organization of Autism Research). Affected children display qualitative impairments in social interaction, expressive and receptive language, and the use of symbolic or imaginative play, with an age of onset before 3 years. In addition, many children display restricted, repetitive, and stereotyped patterns of behaviors. Specific military preparedness issues for families with a child with an ASD include the stress of deployments and frequent moves; early retirement or lack of re-enlistment for family concerns; and limited community resources at many smaller bases. ASDs pose a particularly high level of stress for many military families; however, substantial improvements are attainable through collaborative translational research linked to better care.CORA was initiated in 2006 as a collaboration between Wright-Patterson Air Force Base (WPAFB) and Nationwide Children's

15. SUBJECT TERMS

Autism, Autism Spectrum Disorder, ASD, developmental disability, CORA, chromosomal microarray (CMA), PTEN

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1.0 Summary

In 2006, the Central Ohio Registry for Autism (CORA) was initiated as a collaboration between Wright-Patterson Air Force Base (WPAFB) and Nationwide Children's Hospital (NCH). The primary purpose of CORA was to develop a comprehensive autism registry for genetic studies for military and civilian families in central Ohio. Congressionally-supported funding for the project was secured, beginning in late 2009 (total $2.77 million, September 30, 2009-December 31, 2012) to support (1) development of the CORA registry; (2) expansion of diagnostic and treatment services for WPAFB families through a collaboration with Dayton Children's Medical Center (DCMC); (3) molecular studies to identify novel autism susceptibility genes performed at NCH (Dr. Herman) and The Ohio State University (OSU, Dr. Sadee); and (4) cost and satisfaction analyses for the military services components in Aim 2. Over 260 families have been enrolled in CORA (32% from WPAFB). As part of CORA enrollment, research microarrays have been performed on 86 affecteds (68 from WPAFB). Twelve affecteds have had pathogenic or likely pathogenic variants, including 2 families from WPAFB with large pathogenic unbalanced chromosome translocations. In 2010, a new multidisciplinary autism assessment clinic was established staffed by DCMC psychologists and WPAFB developmental pediatricians. Thirteen multidisciplinary clinics were held and 24 military dependents were assessed. In the molecular research studies, rare variants were identified in 3 out of 5 genes studied in up to 96 registry families. Significant allelic-expression imbalance (AEI) was detected in 13/24 candidate genes tested (54%) and the likely functional variant(s) identified for 3 of these genes. Family based association tests were performed on genotypes obtained for 12 common variants in 7 genes using 115-159 CORA trios. A highly statistically significant association was found for a single-nucleotide polymorphism within the serotonin receptor gene *HTR2A* ($p=0.0003$). Pilot experiments performing whole exome sequencing and RNA transcriptome analysis were initiated and will be continued with new funding under co-operative agreement FA8650-12-26359. Cost and satisfaction analysis of the DCMC/WPAFB multidisciplinary clinics and Social Skills groups was conducted through OSU (Dr. Seiber). Overall parental and provider satisfaction with the multidisciplinary clinics and Social Skills groups were high. Cost analysis of the multidisciplinary clinic determined that it was a self-sustaining model that will be continued and made available as a clinical service for military and civilian families at DCMC.

2.0 Introduction and Background

Autism is the fastest growing developmental disability in the US, costing more than $90 billion/yr, and is, therefore, extremely important to the DoD. According to the Military Times (Robert Dorr, 8/13/11), "autism is the most prevalent special-needs issue in military families." Autism spectrum disorders (ASDs) have a frequency of ~1/88 in the general population (1) and an estimated frequency of 1/88 in the military (Organization of Autism Research). Affected children display qualitative impairments in social interaction, expressive and receptive language, and the use of symbolic or imaginative play, with an age of onset before 3 years. In addition, many children display restricted, repetitive, and stereotyped patterns of behaviors.

It is estimated that genetic factors account for up to 70-80% of the risk for developing an ASD (2). Traditional G-banded karyotyping, subtelomeric fluorescence in situ hybridization (FISH), and fragile X testing identify the underlying cause of an ASD in <10% of cases. Chromosomal microarray (CMA) offers an additional diagnostic yield of 15-20% due to the high sensitivity for detecting submicroscopic copy number variations (CNVs) in the form of deletions and duplications (3, 4). Recently, the importance of rare non-synonymous missense, nonsense, and splice site *de novo* single nucleotide variants (SNVs) has emerged as a result of advances in whole exome sequencing of large research cohorts of ASD families with a single affected individual (5-8). A unifying theme among many of the CNVs identified, as well as susceptibility loci ascertained by linkage, association, and direct gene sequencing studies, is localization of the involved protein at neural synapses (2). While the availability of clinical CMA has greatly improved the diagnosis rates, 70-75% of children with an ASD are still without an identifiable genetic etiology. Understanding the underlying causes of ASDs may help direct current and future medical management, behavioral and physical therapies; predict future outcomes for the child; and provide recurrence risk estimates for the immediate and extended family.

The military Exceptional Family Member Program (EFMP) initiative has resulted in improved services for military families; however, the complexities of ASD diagnoses and treatments, and advances in translational research, including genetics, demand continued efforts to improve the quality of care. Specific military preparedness issues for families with a child with an ASD include the stress of deployments and frequent moves; early retirement or lack of re-enlistment for family concerns; and limited community resources at many smaller bases. ASDs pose a particularly high level of stress for many military families; however, substantial improvements are attainable through collaborative translational research linked to better care.

CORA was initiated in 2006 as a collaboration between Wright-Patterson Air Force Base (WPAFB) and Nationwide Children's Hospital (NCH). The primary purpose of CORA is to develop a comprehensive autism registry for genetic and other studies for military and civilian families in central Ohio. Congressionally-supported funding for the project was secured, beginning in late 2009 (total $2.77 million, September 30, 2009-December 31, 2012). The specific aims of the project are to support (1) development of the CORA registry; (2) expansion

of diagnostic and treatment services for WPAFB families through a collaboration with Dayton Children's Medical Center (DCMC); (3) molecular studies to identify novel autism susceptibility genes; and (4) cost and satisfaction analyses for the military services components in Aim 2. Progress related to each of these aims is detailed below.

3.0 Aim 1 – Central Ohio Registry for Autism Research (CORA)

There are currently 243 active families enrolled in CORA (Table 1), with a total of 915 individuals. Currently, to be eligible for enrollment families must have a child with a previous diagnosis of an autism spectrum disorder (ASD) (Table 2), at least 1 biological parent willing to participate, completed psychological testing that includes the Autism Diagnostic Observation Schedule (ADOS), and genetic testing that includes Fragile X and a genomic oligonucleotide chromosomal microarray (CMA). As noted below, as the clinically recommended standard of care and genetic testing for children with an ASD diagnosis have evolved, so have the enrollment criteria for participation in CORA.

A total of 268 families have enrolled in the study since its inception in 2006. Three families have requested to be removed from the study; 22 additional families were withdrawn due to failure to provide a blood sample on the child with autism and at least 1 biological parent within 1 year of enrollment.

Table 1. CORA Enrollment Statistics

	NCH Families	% NCH	WPAFB Families	% WPAFB	Total Families	Total Individuals
Enrolled	190	71%	78	29%	268	996
Withdrawn	25	100%	0	0%	25	81
Currently Active	165	68%	78	32%	243	915

Table 2. Diagnosis Statistics for Active CORA Families

Diagnosis	Probands Only		Probands & Affected Siblings	
	Male	Female	Male	Female
Autistic Disorder	131 (54%)	24 (10%)	147 (50%)	28 (10%)
Asperger Syndrome	22 (9%)	3 (1%)	25 (9%)	5 (2%)
PDD-NOS	43 (18%)	8 (4%)	54 (19%)	13 (4%)
Autism Spectrum Disorder	11 (5%)	1 (0%)	16 (6%)	3 (1%)
Totals	207	36	242	49

Initially, Fragile X DNA testing and a clinical karyotype were required on the proband for enrollment in CORA. However, as standards of care and practice advanced and CMA replaced a karyotype as part of first tier testing for individuals newly diagnosed with an ASD, criteria to enroll in CORA were similarly updated. To address this issue in cases where insurance, including TriCare, would not provide reimbursement for a clinical CMA, funding was set aside to provide a limited number of research CMAs (up to 100) for participating families. These research arrays were completed in the clinical NCH Molecular Laboratory and are identical to the arrays that individuals receive as a clinical test. Military families were given preference for the research arrays since they have been unable to obtain a clinical CMA due to restrictions in TriCare insurance. In instances where we had lost contact with an enrolled civilian family or the family's insurance prohibited coverage of the CMA, we have been able to provide a limited number of research arrays for non-military CORA families. All of the research array results were reviewed by Dr. Herman (Table 3), and with IRB approval, summary letters were sent to families when we had current contact information for them. In the event that a Variant of Unknown Significance (VUS), Likely Pathogenic, or Pathogenic finding was identified, families were contacted directly by the study coordinator and encouraged to follow-up with a clinical genetics evaluation in their area. Thirteen families with an identified anomaly on CMA have seen Dr. Herman in the NCH Genetics Clinic, one additional family is scheduled to be seen by Dr. Herman in summer 2013 (parent currently deployed), and 3 families have relocated and were seen by clinical genetics departments in their area.

Table 3. Research Microarray Results

Mircoarray Results	Total	Findings
Normal	47	
Benign	5	
Likely Benign	13	
VUS	9	
Likely Pathogenic	8	1q21.1 Duplication 2p16.3 Deletion (+affected sib) 3p26.3 Duplication 3p26.3 Deletion 15q13.3 Duplication 15q11.2 Deletion 16p11.2 Duplication 16p12.3p13.11 Duplication
Pathogenic	3	5p14q Unbalanced Translocation 6q9q Unbalanced Translocation 22q11.2 Duplication
Total Completed	86	

To be eligible for enrollment in CORA, children with an ASD must have also completed psychological testing that includes the Autism Diagnosis Observation Schedule (ADOS). However, this test has only been used clinically in diagnosis for the past few years, and is generally only used during the initial diagnosis process. Therefore, older children may not have had the opportunity to undergo this testing. In an effort to include as many children in the study as possible, a psychometrian was hired to complete the ADOS along with several other psychological tests that may have not been previously completed.

At the start of the study it was felt that the addition of a second research psychological test, the Autism Diagnostic Interview - Revised (ADI-R), would be helpful. The ADI-R is a 2-hour parent interview focusing on areas of communication, social interaction, and restricted/repetitive interests. However, during the second year of the study it became clear that the ADI-R was not providing additional diagnostic information above what was gained from the ADOS and other clinical psychological testing that is routinely completed. Discussion with Dr. Butter at the NCH Autism Center revealed that the ADI-R was not widely utilized in other research studies and was not completed as part of the clinical diagnostic evaluation due to the parental time commitment required. At that point we switched from completing the ADI-R on all children with an ASD, to trying to ensure that all children had the ADOS and current IQ testing completed.

In addition to the clinical genetic testing and ADOS; families are asked to provide a blood sample on all enrolled family members, complete a pregnancy questionnaire, complete a 3-generation family medical history (pedigree), provide consent for release of medical records pertaining to the ASD diagnosis from agencies outside of NCH and the Air Force (AF), and complete pen-and-paper psychological surveys on parents and any unaffected siblings. An extensive, secure database was created using SharePoint and Microsoft InfoPath platforms and utilizing SQL Server for backend data storage. This database was created to store and analyze the information gathered (Table 4). This information is utilized to determine eligibility of families for participation in specific research studies that are conducted as part of the larger grant. To make it easier to identify families that have at least 1 parent enrolled and all required items completed, the designation "SuperSTAR Family" was coined. Since many of the research studies require access to a DNA sample from both parents, we added the term "Complete Trio" to indicate when at least 15ug of primary DNA, and preferably cell lines, are available on the child with an ASD and both biological parents.

Table 4. Enrollment Statistics for Children with an ASD Diagnosis (n=291)

	Complete
Microarray	265 (91%)
ADOS Testing	264 (91%)
IQ Testing	143 (49%)
Genetic Evaluation	158 (54%)
Pregnancy Questionnaire	250 (86%)
Specific Genetic Dx	26 (9%)
Pedigree*	237 (97%)
SuperSTAR Family*	140 (58%)
Complete Trio*	175 (72%)

***Per family (n=243)**

Blood obtained as part of the study is separated into two samples: one from which primary DNA is isolated from whole blood (individuals ≥5 years), and the other is treated to create lymphoblastoid cell lines (all individuals). Lymphoblastoid cell lines provide the opportunity to regrow lines as many times as are need to provide an "unlimited" supply of DNA (or RNA) for research purposes on each enrolled participant. However, the process of cell line creation may introduce novel mutations that are not present in the participant. Therefore, the presence of the primary DNA sample allows confirmation of any molecular findings identified.

A goal of the study is to maintain a 95% success rate in lymphoblastoid cell line creation (Table 5). There are currently 915 individuals enrolled in the CORA study; however, 51 individuals have not completed the blood draw. Several new families have not yet been drawn; several families have young unaffected children where they have requested to wait and combine the research blood draw with a clinical blood draw for another reason; and several parents have failed to follow through with their blood draw after repeated requests. Additionally, at the start of the study it was routine to obtain a sample for cell line creation on the affected child(ren) only. Roughly 10 of these early families have been lost-to-follow-up, and we have only a limited supply of primary DNA on these parents and unaffected siblings. Of the individuals that have provided blood samples, 33 are currently pending completion, and 29 samples have failed and we have been unable to obtain another blood sample for research. We currently have a 96% success rate for lymphoblastoid cell line creation.

Table 5. Cell Line Completion Data

Active Enrolled Individuals	915
Drawn for primary DNA only	49
Not yet drawn	51
Pending	38
Failed	29
Successfully Completed	748 (96%)

Genomics Studies of Developmental and Cognitive Aspects of PTEN Disorders

Over the course of recruiting for CORA, several families have been enrolled where additional clinical testing on the child with autism revealed a mutation within the *PTEN* gene. *PTEN* (phosphatase and tensin homologue deleted on chromosome ten) is a tumor suppressor gene that functions as a cellular lipid phosphatase (9). Mutations in *PTEN* have been identified in a family of related syndromes currently referred to as PTEN hamartoma syndromes (10, 11). These include Cowden syndrome (CS), Bannayan-Riley-Ruvalcaba syndrome (BRRS), and some cases of Proteus-like syndrome (PLS). Individuals with CS typically develop multiple benign harmartomas and have an increased risk of certain cancers, particularly of the breast, uterus, and thyroid. BRRS is characterized by macrocephaly, hamartomas (including lipomas, hemangiomas or intestinal polyps), penile freckling in males and developmental delays. Autism is not a criterion for either CS or BRRS, but there have been several reports of individuals with ASD occurring in families with CS or BRRS (12-14). Subsequently, we and others have described heterozygous *PTEN* mutations in children with ASDs and/or mental retardation/developmental delay (MR/DD) and macrocephaly (15-19). All of the individuals

with *PTEN* mutations and ASD or mental retardation have had significant macrocephaly, often from birth or infancy. At this time it is not clear why some individuals with a *PTEN* mutation will develop macrocephaly plus autism/mental retardation and/or developmental delay, while other individuals may only develop macrocephaly or macrocephaly plus an increased risk of cancer development. Even within a family, development of symptoms can be highly variable among family members.

During the current funding period, Dr. Herman created a "sub-study" within CORA to allow enrollment of individuals with a *PTEN* mutation plus or minus ASD/mental retardation and/or developmental delay. The purpose was to use next generation sequencing to detect secondary or modifier loci that may influence the developmental and cognitive phenotype in children with a *PTEN* mutation. To ensure that we are able to enroll a large enough group of *PTEN* positive individuals (up to 25 families), enrollment was opened up nationwide. Families with children with a *PTEN* mutation and an ASD diagnosis from Ohio are enrolled through the CORA study. Families from outside of Ohio (regardless of ASD status) and families from within Ohio with children with a *PTEN* mutation but without an ASD diagnosis are enrolled through the PTEN study (NCH IRB #10-00138, AF SGE-C Protocol #FSG20120009H). As of December 31, 2012, ten families with a *PTEN* mutation were enrolled; four families enrolled in CORA and six families enrolled through the PTEN study protocol.

4.0 Aim 2 – Delivery of Clinical Services to Wright-Patterson Air Force Base

The aims for Dayton Children's addressed the need to increase clinical services for children with autism as military dependents and within the local community. Our goals were to develop a comprehensive assessment team so that new families coming to WPAFB could have a thorough, one stop evaluation for their children and be enrolled in intervention services sooner. In addition, social deficits are significant challenges for children on the autism spectrum, and few resources were available to address these skills. We developed and implemented a group treatment program for children with autism and social skills deficits. We developed education sessions for parents and provided a session for professionals in the area as well.

Hiring of staff: Dayton Children's Medical Center (DCMC) proposed hiring two post-doctoral fellows, a full time social worker, and one part time office coordinator to support the goals of the project. In addition, licensed psychologists on staff were included on the grant for program development, program implementation and monitoring, supervision and administration. This process was outlined in appropriate monthly reports but as a summary:

- Dr. Lauren Jones was hired as a postdoctoral fellow in December 2009 and has remained with the project. She has been primarily responsible for conducting social skills group sessions, documenting, and recruiting for social skills groups. In addition, she has provided several of the education sessions, and has seen five children in individual therapy/consultation. We were unable to secure a second postdoctoral fellow, although it should be noted that we have been able to address the current patient needs with the staff that was secured.

- Melissa Sears was initially hired as our team social worker, beginning November 2009, but resigned in January 2010. Erin Negri, began with us in 2010 and remains on the grant currently. Ms. Negri decreased her time to .5FTE following the first No Cost Extension.

- Dr. Greenwell was identified as a staff psychologist to assist with the grant. Dr. Greenwell resigned from the hospital in November 2010. Responsibilities were managed by Dr. DeWitt subsequently.

Establishment of multidisciplinary assessment clinic: The Comprehensive Autism Assessment Team (CAAT) was established in 2010, and clinics have been offered every other month to military families. Represented disciplines include developmental pediatrics (WPAFB), psychology, speech therapy, and occupational therapy. Children are assessed in several domains during the appointment, with a summary and recommendations provided to parents before leaving. Three medical residents have been able to attend the CAAT clinic and have found this to be an excellent venue for multidisciplinary training.

- We have met for a total of 13 clinics and have assessed 24 children.

- Five clinics were cancelled because of lack of referrals or scheduling conflicts.

After a review of the CAAT clinic, Dayton Children's Medical Center has decided to continue this service and open it up to local civilian families in addition to military families. The multidisciplinary model developed through the grant has proven to be a comprehensive, efficient, and self-sustaining method for assessment of autism spectrum disorders.

Development of Alternative Scheduling for WPAFB families: Beginning in October 2009, time was protected each week for families needing services through DCMC. Families utilizing this protected time were often referred for updated psychological evaluations or testing where a prior diagnosis was already in place and a full CAAT clinic assessment was not needed. This allowed for timely scheduling when referrals were received from the developmental pediatricians on base.

Development and Offering of Education sessions: We proposed offering nine sessions per cycle year. Twenty-four sessions were offered (Table 6), 15 were provided, and 9 sessions were cancelled due to lack of enrollment. Attendance increased over time and fewer sessions were cancelled. Advertising for these sessions allowed for increased networking with other professional organizations within the community. Many of these agencies began to offer similar sessions of their own in the community, allowing for the development of increased community opportunities.

Table 6. Education Topics Years 1-3

Education Topic Year 1	Date of Offering	Attendees	Military
Introduction to Autism	January 10, 2010	None	None
Toilet Training	February 8, 2010	5	1
Behavior Management	March 8, 2010	7	2
Building New Skills	April 5, 2010	None	None
Building Social Skills	May 10, 2010	None	None
Community Resources	June 14, 2010	None	None
Medication Options	July 12, 2010	None	None
Adolescence	August 9, 2010	8	4
Parent/Professional Panel	September 13, 2010	None	None
Total Year 1		20	7

Education Topic Year 2	Date of Offering	Attendees	Military
Transition Planning for Teens	January 10, 2011	8	3
Behavior Management	February 14, 2011	13	1
Toilet Training	March 14, 2011	12	2
Functional Behavior Assessment/Positive Behavior Supports	April 11, 2011	5	2
Building Social Skills	May 9, 2011	10	3
IEP Planning	June 13, 2011	None	None
Parent Panel	July 11, 2011	None	None
Medication Options	August 8, 2011	None	None
Advocacy	September 12, 2011	6	2
Total Year 2		54	13

Education Topic Year 3	Date of Offering	Attendees	Military
Behavior Management	February 13, 2012	20	0
Transition Planning for Teens	March 12, 2012	6	3
IEP planning for school aged children	April 9, 2012	14	5 (retired)
Toilet Training	May 14, 2012	12	0
Behavior Management	October 8, 2012	12	0
IEP Planning	November 12, 2012	12	0
Total Year 3		76	8

Development and Implementation of Social skills groups:

Social skills groups for children with ASDs were lacking in the community and identified as a significant need for children with autism spectrum disorders. A total of 12 groups were completed over the course of the grant (Table 7). Topics and handouts were provided with the monthly reports, but are also summarized in Appendix A. A total of 65 children participated in these groups, 19 children did not meet criteria for enrollment, 3 of whom were military, and 27 children indicated no interest following referral or did not complete paperwork (12 military).

Table 7. Social Skills Group Attendance

Groups Offered	12
Total Children Served	65 (49 boys, 11 girls)
Mean Age	10.57
Military Dependent	33 (51%)

Fifty-four parents completed evaluation forms from the Social Skills Groups (Table 8) with the following results noted on a five point scale (five being "very good):

Table 8. Parental Satisfaction Rating of Social Skills Groups

	Mean Rating
Facilities	4.54
Time of session	4.52
Usefulness of Child Handout	4.65
Usefulness of Parent Handout	4.70
Ease of Homework	4.43
Benefit of Homework	4.43

Reunion sessions were offered to allow children to meet again and socialize with other group members. Parents had also consistently noted on feedback forms that they wished groups lasted longer. Six reunion sessions were offered for a total of 49 attendees.

With the approval of the second No Cost Extension, we were able to have the opportunity to respond to previous parental comments about wanting services to last longer. We invited all military families that had previously completed a Social Skills Group to return for a series of weekly social skills topic sessions (Table 9). We had hoped to revisit many of the topics presented during group to review the content and allow for added discussion and clarification.

Parents could choose to allow their child to attend all or just some of the sessions as they were not designed to be sequential.

Table 9. Weekly Social Skills Topic Sessions

Topic	Date of Offering	Attendees	Mean Age	Parent Overall Satisfaction
Choosing and Maintaining Friendships	October 9, 2012	4	13	5
Bullying	October 16, 2012	4	12.75	5
Anger Management	October 23, 2012	5	13	5
Personal Responsibilities and Group Projects	November 6, 2012	5	14.40	5
Unwritten Social Rules at Home	November 13, 2012	5	14.40	5
Unwritten Social Rules at School	November 20, 2012	6	13.67	5
Internet Safety	November 27, 2012	6	13.80	5
Sibling Issues (for sibs)	December 4, 2012	Canceled due to lack of enrollment		

Development of family resource directory: We were able to develop a Family Resource Directory that is provided to families at the time of diagnosis and upon request. Due to the dynamic nature of information contained in the directory, it is monitored and updated on a regular basis.

Development of curriculum for workshop for professionals and educators. The medical professional and educator workshop sessions were developed and offered during the second year of the grant.

- The professional conference was held on May 6, 2011, entitled "Surveillance and screening in autism spectrum disorders", featuring Diana Robins, Ph.D. and Daniel Coury, M.D. Overall satisfaction with the conference was good to very good (Table 10).
    - Forty-eight practitioners were in attendance (27 physicians, 2 medical residents, 10 nurses/nurse practitioners, 9 other).

Table 10. Medical Professionals Conference Satisfaction Rating

Objectives	Physician Average Rating	Non-Physician Average Rating	Total Average Rating
Identify Common warning signs of autism	4.73	4.69	4.72
Identify methods of screening for ASD	4.85	4.69	4.79
Understand and administer the MCHAT	4.76	4.77	4.76
Differentiate surveillance from screening and discuss new research interests	4.92	4.85	4.89

- The Educators Conference, entitled "Educational strategies for children with autism spectrum disorders", was held on September 17, 2011. Overall satisfaction with the conference was good (Table 11).
    - Eighty-one professionals registered, 51 attended, and 49 completed ratings. Twenty-one regular education teachers (41%), nine intervention specialists (18%), five principals (11%), five aides (11%), three school counselors (6%), three speech therapists (6%), and four parents (9%) attended.
    - Five counties were represented (Montgomery, Greene, Miami, Darke, Preble) with the majority of attendees being from Montgomery county (47%). Eighteen school districts/private schools were represented.

Table 11. Educational Professionals Conference Satisfaction Rating

Objective	Average Rating
Learn behavioral teaching strategies and best practice for teaching students with autism spectrum disorders	4.52
Learn strategies for incorporating IEP goals and district standard into daily teaching/evaluation across settings	3.93
Learn strategies for functional behavior assessment and positive behavior support for learning self-regulation	4.51

5.0 Aim 3a – Identifying Genetic Variation using Allelic Expression Imbalance (AEI) (Dr. Sadee Lab)

The original aims for the molecular studies were revised with the approval of a no cost extension from September 30, 2011 to September 29, 2012, to take advantage of the dramatic advances in DNA sequencing technology. Progress toward the revised aims are summarized here.

Preparation of autopsy brain tissues. We obtained and extracted DNA and RNA from a large number of prefrontal cortex tissues (>200); a subset were derived from deceased subjects diagnosed with ASD. Complementary DNA (cDNA) libraries were constructed for 56 brain tissues in the autism cohort (21 ASD, 15 first-degree relatives of ASD patients, 20 controls).

Allelic Expression Imbalance (AEI) analysis in candidate genes (Milestones 1 and 2). Our goal was to identify ASD candidates genes that carry frequent regulatory polymorphisms (rSNPs) with high likelihood of affecting clinical traits. Using SNaPshot technology (20-23), we screened 24 candidate genes in cDNA made from the ASD brain autopsy tissues and controls (Table 12) for the presence of AEI. For *CHRNA5*, six frequent single nucleotide polymorphisms (SNPs) in complete linkage disequilibrium showed a >2.5 allelic expression difference (24). Four additional genes listed in Table 1 (*CNTNAP2*, *SLC1A1*, *HTR5A*, and *HTR2A*) demonstrated significant AEI, suggesting the presence of regulatory variants affecting mRNA expression in prefrontal cortex (PFC) tissues. Rare copy number variations (CNV) and mutations, as well as association studies have previously implicated *CNTNAP2* in ASDs (25, 26). The serotonin 2A receptor gene (*HTR2A*) and the neuronal glutamate transporter gene (*SLC1A1*) were studied in greater detail, as outlined below. For *SLC1A1*, it appears that at least two regulatory variants of different effect sizes are present, one less common variant with major allelic mRNA expression differences and a second more common variant of smaller effect (see below).

Table 12. List of genes analyzed for allelic mRNA expression imbalance in PFC tissues

Gene symbol	C_T Average	SNPs / Samples screened	AEI-positive*	AEI Range*
Cellular adhesion and synapse structure				
CNTNAP2	25.4	1/25	**2 - 4**	0.81 – 1.22
CNTN4	33.5	Gene expression too low		
DLG4	23.5	1/19	1	0.70 – 1.10
EPB41L1	27.5	1/16	2	0.93 - 1.13
NRCAM	24.6	3/39	1	0.77 – 1.82
PCDH9	26	1/29	0	0.75 – 1.22
Glutamate and GABA signaling				
GABRB3	29.2	Gene expression too low		
GAD1	28.2	1/16	Still under analysis	
GRIA2	26.6	1/13	1	0.90 – 1.36
GRM5	28.7	1/10	0	0.89 – 1.11
SLC1A1	28.6	3/42	**14**	0.64 - 2.51
SLC1A2	29.1	1/17	0	0.89 - 1.06
SLC6A1	28.6	1/24	0	0.91 – 1.01
Alternative splicing and RNA processing				
A2BP1	N/A	1/22	1	0.81 – 1.36
HNRNPA3	22.7	2/24	0	N/A
NCBP2	23.8	1/20	0	0.92 – 1.08
NOVA1	28.8	1/18	Still under analysis	
SF4	28.9	1/12	Still under analysis	0.44 – 1.61
SNRPN	Analyzed in non-ASD samples using SOLiD sequence, need to revisit in ASD samples			
General risk genes and therapeutic targets				
CHRNA5	30.1	2/47	**27**	0.62 – 7.6
HTR2A	25.1	9/59	**32**	0.90 – 2.2
HTR5A	26.9	4/42	**14**	0.90 – 1.70
HTR6	33	Gene expression too low		
SLC25A12	25.2	1/24	0	0.89 – 1.21

** - AEI range represents allelic mRNA ratios adjusted to allelic genomic DNA ratios set to 1. Any deviation from unity suggests the presence of regulatory variants (highlighted in red). Samples are designated AEI-positive if their AEI is outside of ± 3 standard deviations of the assay for the entire population*

Deep sequencing of the transcriptome and genome. In year 2 of the grant and continuing through the no-cost extension, we modified our experimental approach by expanding the search for candidate genes genome-wide. Dr. Sadee secured an NIH U01 grant with funds available for advanced next generation sequencing (up to 200 giga-bases per run, enabling whole genome sequencing) and completed studies validating our ability to reliably detect AEI on a genome-wide basis (manuscript in preparation). Validation of this method was a crucial step for establishing the reliability of this technology to detect AEI in the autism cohort and represents an exponential leap forward in our ability to find functional genetic variants in ASD. The first whole-transcriptome studies of 3 ASD tissues and 1 control measured allelic expression imbalance in 2061 total genes (3522 total instances measured), identifying 20 genes (21 instances) where AEI was 3-fold or greater (Table 13) as high priority targets for harboring functional genetic variants potentially contributing to ASD. Of relevance, 19 of the 21 significant AEI measurements are in ASD samples, while only 2 are in the control sample. Of the 20 genes, two are already implicated in ASD, both regulating synaptic function and

plasticity, while others are biologically plausible candidates that affect synaptic function but have yet to be associated with autism. The neuronal calcium sensor – 1 gene (*NCS1*) displayed 3.1-fold AEI in an autistic sample and was found in an autistic individual to harbor a non-synonymous mutation that affects structure and function of the protein, possibly contributing to ASD risk (27). The second gene, regulating synaptic membrane exocytosis 3 (*RIMS3*), also displayed 3.1-fold AEI in an autistic sample and was identified as an ASD risk gene through discovery of a *de novo* microdeletion in an autistic patient (28). Moving forward, we are applying this approach to the remaining ASD samples and subsequently validating our findings with traditional methods – providing an unprecedented novel path towards understanding the genetics of ASD.

Table 13. RNA-Seq Genes with AEI

Gene Symbol	Function	AEI	Sample
AKAP5	Anchors PKA at post-synaptic densities	3.25	ASD-1
CHML	Rab geranylgeranylation	4.45	ASD-1
CNR1	Cannabinoid receptor	3.79	ASD-3
DGUOK	Phosphorylation of mitochondrial purine deoxyribonucleosides	3.06	ASD-2
DUSP6	Protein tyrosine phosphatase	3.34	ASD-1
FEM1B	Induction of apoptosis	3.17	Control
GATC	Mitochondrial translation	4.21	ASD-2
HINT3	Ribonucleotide hydrolase/transferase	3.38	ASD-3
*NCS1**	Calcium-dependent GPCR phosphorylation at synapse	3.13	ASD-1
PARG	Catabolism of poly(ADP-ribose)	4.13	ASD-2
PCDH7	Cell-cell adhesion in brain	3.09	ASD-2
*RIMS3**	Calcium-dependent exocytosis at synapse	3.11	ASD-3
SLC7A1	Cationic amino acid transporter	3.28	ASD-2
SOCS5	Suppressor of cytokine signaling	4.00	ASD-3
TAF7	TATA box transcription regulator	3.83	Control
TAF15	TATA box transcription regulator	3.46	ASD-2
TIMM23	Mitochondrial transport of transit peptide-containing proteins	3.48-4.00	ASD-2&3
TMEM167A	Unknown transmembrane protein	3.25	ASD-1
TPM1	Cytoskeleton structure and cell adhesion	3.13	ASD-2
ZBTB1	Regulator of transcription	3.08	ASD-2

*Previously implicated in ASD

Identify functional SNPs in strong candidate genes (Milestone 3). We conducted detailed studies with one of the major serotonin receptors (*HTR2A*), discovering novel transcripts in the prefrontal cortex (PFC). The function of one SNP related to the formation of a newly identified splice variant, previously implicated in clinical associations studies in multiple CNS disorders, was indeed affecting *HTR2A* splicing and function, as shown *in vitro* with reporter-gene

experiments and *ex vivo* in the autopsied tissues (29). Hundreds of genetic association studies have implicated *HTR2A* as a candidate gene in numerous central nervous system (CNS) disorders, including ASD. Moreover, the serotonin 2A receptor is a main target of atypical antipsychotics, and therefore of high importance as a potential pharmacogenomics biomarker. Yet, the functional polymorphisms and underlying mechanisms have remained unknown so that association studies have employed tenuous surrogate marker SNPs in the gene locus to study associations with clinical phenotypes. We therefore decided to address this question in detail, investing extraordinary effort in resolving the molecular genetics of *HTR2A*. A first set of analyses identified three *HTR2A* splice variants, one of which has never been described ($E2^{tr}$; Figure 1), and characterized their effects on gene expression. Even though previous clinical association results strongly suggested the presence of regulatory variants, no AEI was detectable in the known *HTR2A* transcripts, except in one African-American subject, where we identified a likely causative SNP that significantly affects splicing. Subsequently, it became evident only from the RNAseq data (Figure 2) that HTR2A transcription in the PFC extends beyond the known annotated 3' and 5' untranslated regions (UTR). The 1 kb novel 5' UTR contains a SNP frequently used in clinical association studies (rs6311, minor allele frequency ~45%), often with positive phenotype associations. Using a variety of molecular genetic techniques, including qPCR, cloning of the 5' UTR into expression vectors, and reporter gene assays measuring AEI and luciferase expression in human cell lines, Dr. Smith showed that rs6311 directs ~2-fold decreased formation of *HTR2A* transcripts with the long 5' UTR. He also demonstrated that the longer 5' UTR has higher translation efficiency, implicating rs6311 as a "loss-of-function" allele indirectly at the protein level and directly at the RNA level.

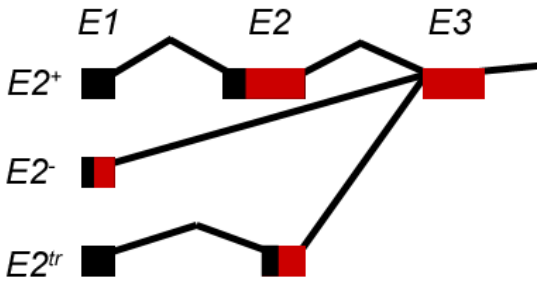


Figure 1. Alternative Splicing of *HTR2A*. Three mRNA transcripts differing at exon 2 are found, including the full-length isoform (E2+), a truncated isoform with a different protein translation start site (E2-) indicated by red shading, and a novel spliceoform in which exon 2 is truncated (E2tr).

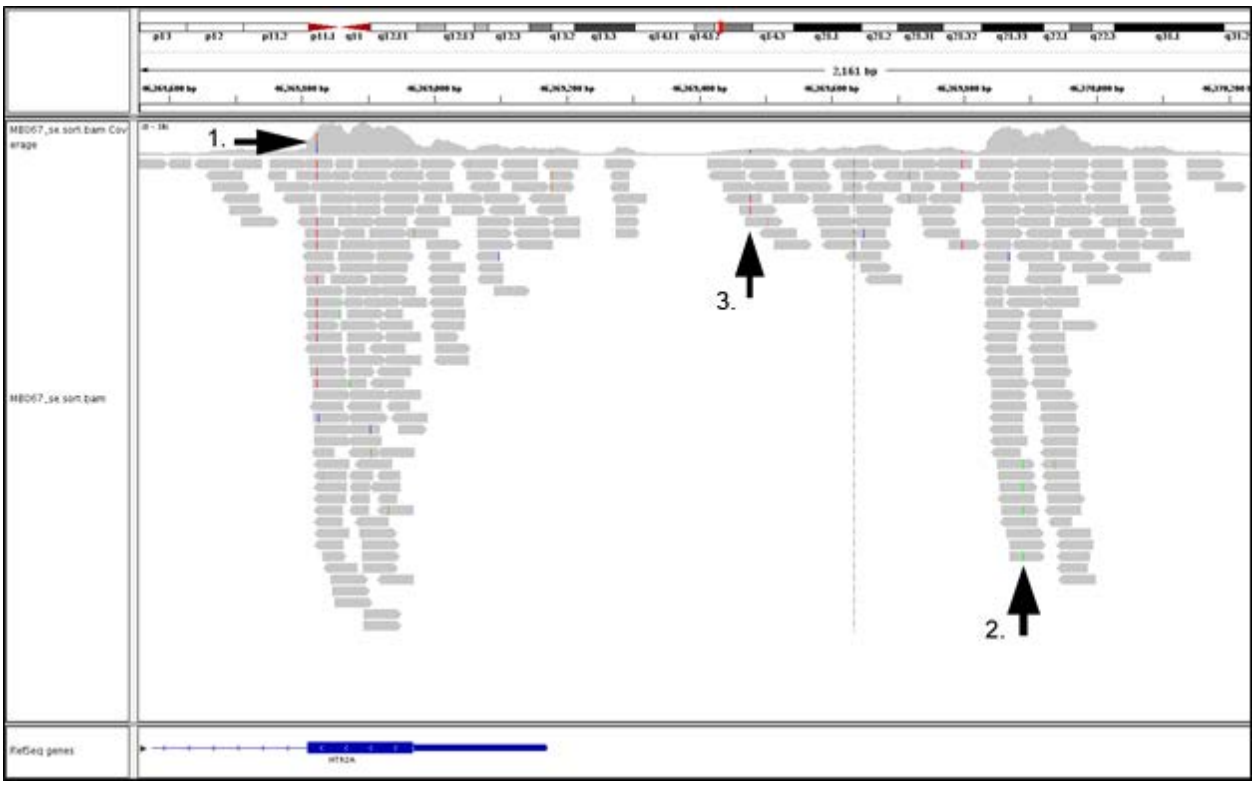


Figure 2. RNAseq alignments of *HTR2A* displaying extended 5'UTR coverage. Allelic expression in the annotated gene region (arrow 1; rs6312) shows no allelic imbalance (12 wild-type alleles *versus* 12 SNP alleles), whereas a SNP in the 5'UTR (arrow 2; rs1328685) shows major allelic differences (17 wild-type *versus* 4 SNP alleles), caused by rs6311 (arrow 3).

Expression across rs6311 genotypes measured in our brain samples is shown in Figure 3. This finding significantly advances our understanding of the genetic influence of *HTR2A* on CNS disorders and drug response, as we demonstrated the haplotype structure of functional alleles in *HTR2A* significantly contributes to depression severity. Dr. Herman's group has already genotyped this SNP in the CORA cohort, finding a significant difference in allele transmission from parent to affected offspring with ASD (see below). We are encouraged with this result. Further work will focus on identifying the transcription factor binding at the rs6311 SNP location; one strong candidate (*TCF4*) is implicated in schizophrenia through genome-wide association studies (GWAS) at the level of genome-wide significance (30).

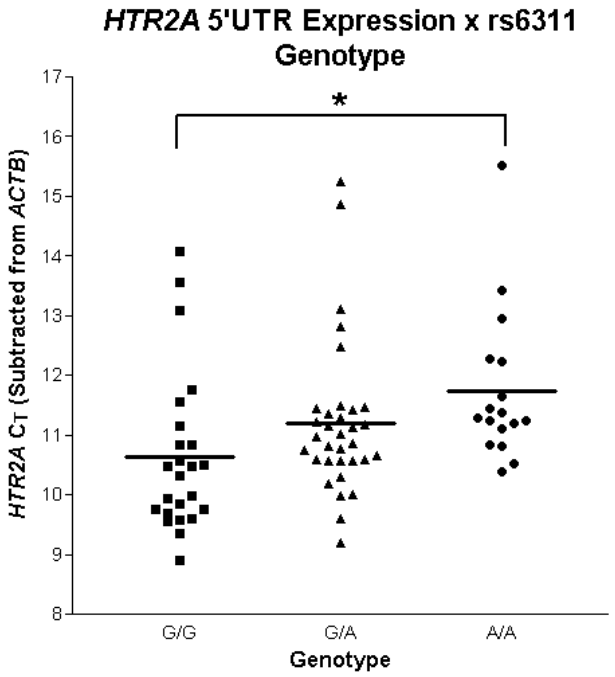


Figure 3. Extended 5'UTR Expression Across rs6311 Genotypes. Homozygous rs6311 "G" allele carriers express 2-fold more upstream 5'UTR mRNA *versus* homozygous "A" allele carriers, as measured via qPCR in postmortem PFC tissues. *p<0.05. A lower threshold cycle (C_T) indicates higher expression, ie, G/G>G/A>A/A.

Progress in functional SNP identification for SLC1A1 and HTR5A. We have identified a SNP in *SLC1A1* correlated with allelic expression. *SLC1A1* modulates the amount of glutamate in the synapse and therefore is in a critical focal point of neurotransmission. *SLC1A1* displays biphasic allelic differences, in which there is a strong but less frequent AEI and a more frequent but subtle AEI. We have primarily focused on the strong AEI, as we hypothesize that it will have a greater effect on clinical phenotype. For the more robust AEI, 3 of 42 samples informative for allelic expression display a 1.5 to 2.5-fold allelic difference. Twenty-four SNPs have been genotyped in all samples and correlated with the major allelic difference. Two SNPs, rs12682807 and an unannotated SNP from the 1000 Genomes Project, best correlate with allelic expression; however neither SNP explains the allelic differences perfectly. rs12682807 was added to the CORA genotyping panel, as an exhaustive search revealed no other SNPs better explaining AEI.

Allelic expression for *HTR5A* measured via SNaPshot indicates the presence of a frequent strong *cis*-acting regulatory variant responsible for directing 1.3 to 1.7-fold allelic differences, measured at four different marker SNPs (rs6320, rs1800883, rs1371817, rs1631327). Fourteen of the 42 brain samples analyzed are positive for allelic differences, suggesting that the functional variant has a minor allele frequency of at least 30% in our samples. SNP scanning has ruled out 11 SNPs

as likely functional variants, although haplotype structure within this region as indicated by these SNPs are directing us to other promising candidates for further genotyping.

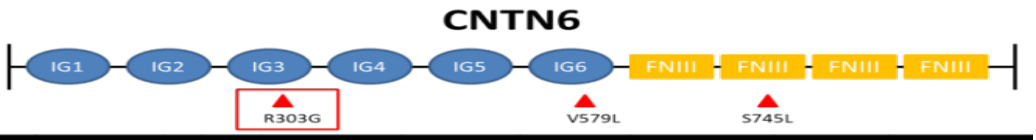
Clinical Association Studies in CORA Cohort (Milestone 4). The molecular genetic studies here, together with our previously identified functional variants, yielded 10 SNPs in 7 genes for clinical association studies. This milestone was moved to Aim 3B to be completed by Dr. Herman.

6.0 Aim 3B – Candidate Gene Sequencing (Dr. Herman Lab)

Candidate gene sequencing. Sequencing by other groups of coding regions of candidate genes involved in neurotransmission, CNS neurodevelopment, and encoding synapse proteins have resulted in the identification of numerous genes newly implicated in ASD. In years 1 and 2 of the grant, we performed sequencing of 5 candidate genes – *CNTN4, CNTN6, NSDHL, PTPRG,* and *PTPRD* – in up to 95 affecteds in CORA for each gene.

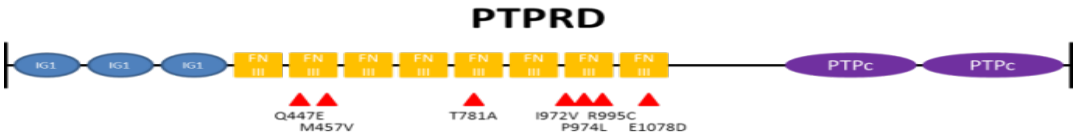
CNTN4 and *CNTN6* encode contactins that are structural proteins involved in complexes at the synapse. They are located together at the distal tip of chromosome 3p26.3. They were chosen for sequencing based, in part, on recent data linking the related genes *CNTNAP2* and *CNTN3* with autism. More importantly, Dr. Herman identified a patient with severe autism and a ~500 kb deletion of 3p26 detected by microarray that included non-coding exon 1 of *CNTN4* and 3' coding exons of *CNTN6.* Unique heterozygous missense variants in *CNTN4* were identified in 4/75 unrelated individuals with an ASD sequenced from CORA, as well as in 1/107 controls. All of the variants were inherited from an unaffected parent. All of the amino acid substitutions were non-conservative, occurred at evolutionarily conserved positions, and were, thus, felt likely to be deleterious. Although additional, larger studies will be necessary to confirm these results; our data suggest that *CNTN4* may function as an autism susceptibility locus in combination with other genetic and/or environmental factors. These results were published in 2011 (31). They were also presented by Col. Randall Zernzach, WPAFB, at the annual Military Healthcare Conference, January 2011 as a plenary talk. Several subsequent publications by other groups have included *CNTN4* in the list of candidate autism susceptibility genes.

The exons and splice junctions of *NSDHL, CNTN6, PTPRG,* and *PTPRD* were sequenced in 96 affected individuals using specific primers and standard Sanger sequencing techniques (Figure 4). No potentially pathogenic variants were found in *NSDHL*, an enzyme involved in cholesterol biosynthesis that was first described by the principal investigator (PI). Three rare non-synonymous variants were found within the coding exons of the *CNTN6* gene. Two of these variants were predicted to be potentially damaging to the structure of the protein encoded by the gene. One of these damaging variants (R303G) lies within a region found to be important for the binding of CNTN6 to the PTPRG protein, a protein tyrosine phosphatase that interacts with contactins. Two rare variants, and one common variant, were found within exons of the *PTPRG* gene itself. An additional novel variant that could affect transcription was found within the 5' UTR of the gene. The two rare coding variants were predicted to be damaging to the protein structure.



Exon	rs#	Ref	Obs	Amino Acid Δ	Codon Δ	# Affected	1000 Genomes MAF	Polyphen Score	Polyphen Prediction
8	rs41293401	G	A	R303G	CGA→CAA	3/96 (2sibs)	0.008	0.984	Probably Damaging
14		G	T	V579L	GTA→TTA	1/96	0	0.986	Probably Damaging
18		C	T	S745L	TCG→TTG	1/96	0	0.183	Benign

Exon	rs#	Ref	Obs	Amino Acid Δ	Codon Δ	# Affected	1000 Genomes MAF	Polyphen Score	Polyphen Prediction
1		C	G	5' UTR	N/A	1/96	0.002	N/A	N/A
1		C	A	P7T	CCG→ACG	1/96	0	0.978	Probably Damaging
3	rs62620047	T	C	Y92H	TAT→CAT	34/157	0.087	0.0	Benign
9		G	T	E371D	GAG→GAT	1/96	0	0.938	Probably Damaging



Exon	rs#	Ref	Obs	Amino Acid Δ	Codon Δ	# Affected	1000 Genomes MAF	Polyphen Score	Polyphen Prediction
20	rs10977171	C	G	Q447E	CAG→GAG	1	0.05	0.117	Benign
20		A	G	M457V	ATG→GTG	1	0	0.023	Benign
25	rs72694737	A	G	T781A	ACT→GCT	1	0.012	0.003	Benign
27		A	G	I972V	ATT→GTT	1	0	0	Benign
27		C	T	P974L	CCA→CTA	1	0	0	Benign
27	rs35929428	C	T	R995C	CGT→TGT	41/160	0.056	0.007	Benign
29	rs7869444	G	T	E1078D	GAG→GAT	1	0.008	0	Benign

Figure 4. Summary of variants identified in candidate genes *CNTN6, PTPRG,* and *PTPRD*.
Rs# refers to reference number in dbSNP database. Ref=expected wild type base. Obs=variant (always heterozygous). MAF=minor allele frequency in 1000 Genomes database. Polyphen is a tool that predicts the possible consequences of amino acid substitution based on physical and comparative attributes of the amino acid.

Finally, *PTPRD* was studied because of a small intragenic deletion found by microarray in another ASD patient evaluated by Dr. Herman. Seven non-synonymous variants were found within the coding regions of the PTPRD gene, three of which were novel. None of these variants was predicted to be damaging to the tertiary protein structure. All of the previously reported variants were found at expected frequencies in CORA (no transmission disequilibrium) (Figure 4). A manuscript is in preparation describing our findings of rare variants in *CNTN6* and *PTPRG*.

Association studies using a validated SNP genotyping panel developed by Dr. Sadee. We have performed association studies using trios in CORA for SNPs identified by Dr. Sadee (Aim 3a) or others that he has validated as showing significant AEI. As shown in Table 14, most of the SNPs involve pathways for neurotransmitters relevant to ASD.

Table 14. Panel of Validated SNPs in Key Candidate Genes for CORA Association Studies

Gene	SNP	Function
HTR2A, serotonin transporter 2A	rs6311	Allelic 5'UTR expression, translation
HTR2A	rs6314	Allelic 5'UTR expression
SLC1A1, glutamate transporter 1A1	rs12682807	Major allelic mRNA expression
CHRNA5, nicotinic α5 receptor subunit	rs880395	Allelic mRNA expression (enhancer)
CHRNA5	rs16969968	Loss-of-function protein variant (D398N)
DRD2, dopamine receptor D2	rs2283265	D2S/D2L splicing
DRD2	rs12364283	Allelic mRNA expression (promoter SNP)
SLC6A3 (DAT), dopamine transporter	rs6347	Allelic mRNA expression
SLC6A3 (DAT)	rs27072	Allelic mRNA expression
SLC6A3 (DAT) (VNTR)	rs3836790	Allelic mRNA expression
MAOA, monoamine oxidase A	rs2064070	mRNA expression (allelic and *cis*-eQTL)
TPH2, tryptophan hydroxylase 2	rs7305115	mRNA splicing/frameshift, allelic expression

Association studies have now been performed for all 12 of these polymorphisms (11 SNPs and 1 varaiable nucleotide tandom repeat (VNTR) polymorphism) in 115-159 trios in CORA (Table 15). SNPs were analyzed using commercially available fluorescent-based TaqMan® PCR assays (Life Technologies). Analysis of the VNTR utilized fluorescently labeled custom primers with PCR fragments separated according to size on an ABI Genetic Analyzer (Sadee lab). A statistically significant association was found for the SNP rs6311 within the serotonin receptor gene *HTR2A* (p=0.0003) with over-transmission of the more common allele (Table 15). As discussed above, this allele demonstrates higher RNA and protein expression in assays performed in the Sadee lab. These findings could have future implications, if replicated, for treatment with atypical antipsychotics that target HTR2A. More importantly, these results confirm our ability to demonstrate significant associations in a relatively small, but well-phenotyped cohort using validated functional SNPs of relatively high frequency (minor allele frequency >0.2-0.3).

We also detected potentially significant associations with 2 subphenotypes – ADHD with rs6311 in HTR2A and macrocephaly with rs880395 in CHNRA5. However, these data will require further analyses. We are also establishing outside collaborations to replicate these findings in additional autism cohorts.

Table 15. Transmission Disequilibrium Test Results for 12 Biologically Functional Variants in CORA Trios

Gene	dbSNP ID	Minor Allele	Major Allele	HapMap CEU MAF	Trios Total	TDT Allele	T	U	Chi Sq	p-value	Permuted p-value†
HTR2A	**rs6311**	**A**	**G**	**0.465**	**159**	**A**	**58**	**104**	**13.22**	**0.0003**	**0.0042**
HTR2A	rs6314	T	C	0.062	159	T	34	28	0.58	0.4461	0.9989
SLC6A3	rs6347	G	A	0.248	159	G	65	45	3.636	0.0565	0.5184
SLC6A3	rs27072	A	G	0.179	159	A	43	42	0	0.9136	1
SLC6A3	rs3836790	*	*	N/A	157	**other	57	45	1.412	0.2348	0.9535
DRD2	rs2283265	T	G	0.167	159	T	36	44	1.532	0.3711	0.9972
DRD2	rs12364283	C	T	0.084	159	C	17	19	0.2571	0.7389	1
MAOA	rs2064070	T	A	0.272	159	A	28	33	0.1525	0.5221	1
TPH2	rs7305115	A	G	0.357	159	A	85	69	1.684	0.1973	0.9343
CHRNA5	rs880395	A	G	0.367	115	A	53	59	0.2252	0.5708	1
CHRNA5	rs16969968	A	G	0.385	115	A	46	47	0.0989	0.9174	1
SLC1A1	rs12682807	C	A	0.115	115	C	18	23	0.6098	0.4349	0.9988

* rs3836790 is a variable number tandem repeat (VNTR) polymorphism with multiple alleles

** TDT analysis was performed comparing the more common 6 repeat allele against all other alleles

† corrected p-value after 10,000 permutations

"T" represents Transmitted allele; "U" Un-Transmitted allele

Exome sequencing of selected CORA families. With the development and rapid implementation of next-generation sequencing technology, we were able to perform exome sequencing on selected individuals from 39 families (132 total samples). The samples included 6 families with a rare variant (or deletion) involving *CNTN4*; 20 trios with both parents and the affected child from families with a single affected individual (sporadic cases); 9 multiplex families where more than a single child is affected; and 4 families in which one or more family members have a mutation in the PTEN gene that has been associated with macrocephaly and ASDs and/or intellectual disability (18, 19). For families with a *CNTN4* or *PTEN* mutation, we were looking for changes in additional (modifier) genes that might affect the phenotype. In sporadic families, we were searching for de novo pathogenic variants as found by other groups within the past year (32-36). In the multiplex families, we were looking for shared inherited variants in affected siblings or a major effect from a single X-linked or autosomal recessive gene mutation.

Exome capture and sequencing were performed in collaboration with the research institute Biomedical Genomics Core (BGC), Dr. Peter White, Director. Again, due to rapid technological advances, exon capture was performed using several different kits - Agilent SureSelect Human All Exon 38Mb kit (some *CNTN4* samples); Agilent SureSelect 50Mb kit (some *CNTN4* and *PTEN* samples); Illumina 50 Mb kit (some *PTEN* samples); and Agilent SureSelect 70 Mb kit (all remaining samples). The Illumina kit had a high percentage of off-target (non-exonic reads) making the data very noisy and difficult to interpret. The 50 Mb Agilent kit contains additional coding exons compared to the 38 Mb kit, while the 70 Mb kit contains primarily additional non-coding regions.

Exome sequencing was performed on a state-of-the-art Illumina HiSeq 2000 Genome Analyzer, purchased by Dr. White with an National Institute of Health (NIH) capital equipment grant. Currently, all samples are prepared with the Agilent 70 Mb exon capture kit and loaded 3-4 bar-coded samples per lane. Sequencing is performed to a depth of at least 50x coverage (average 60-75x) with 100 bp paired-end reads. Improved software for data analysis (see below) and the quality of the data with the read depth >50x on the Agilent 70 Mb kit enabled analysis, for the first-time, of small insertion/deletions (INDEL), as well as SNPs.

A summary of the total number of samples that have been subjected to exome sequencing is shown in Table 16**.** One additional trio from a sporadic family was subjected to exome sequencing; however, non-paternity was detected and the family could not be analyzed. We are currently examining options and costs to perform paternity/identity testing on all families prior to exome sequencing. In addition, due to the poorer quality of some of the early sequence data for the *CNTN4* and *PTEN* families, these samples will be re-run in the new grant using the Agilent 70 Mb exon capture kit and the current BGC analysis pipeline.

Table 16. Exome Sequencing Summary

Group	Families	Individuals
Sporadic	20	60
Multiplex	9	38
CNTN4	6	22
PTEN	4	12
Total	39	132

Data analysis is a critical component of next generation sequencing, and our BGC has spent considerable effort developing an efficient, comprehensive analysis pipeline. A summary of the current procedures used by the BGC are provided here. Acquisition of data from the Illumina HiSeq 2000 uses Genome Analyzer System Software developed by the manufacturer. Primary analysis (image processing, base calling, and quality control) is performed via the HiSeq Control Software and Real Time Analysis (Illumina RTA 1.13.48). Consensus Assessment of Sequence and Variation software (CASAVA 1.8.2) then performs sample demultiplexing and generation of quality control metrics, including error statistics and diagnostic plots which are used to assess data quality. Secondary analysis is the process of sequence alignment and variant calling. Dr. White's laboratory has spent over 18 months developing and optimizing Churchill, a fast and

comprehensive data analysis pipeline for the discovery and annotation of genetic variation, along with methods for candidate gene prioritization (Figure 5). The pipeline utilizes the Burrows-Wheeler Aligner (BWA), Picard tools, and the Genome Analysis Toolkit (GATK) (37). These steps perform the alignment to a reference human genome sequence (currently hg19), local realignment, PCR duplicate sequence marking, base quality score recalibration, allele calling, improved SNP calling, SNP prioritization, small INDEL detection, and the generation of error statistics and diagnostic plots that are used to assess data quality. For our exome capture data sets, variant call files (VCF) are generated using the GATK's Unified Genotyper. It uses a Bayesian genotype likelihood model to estimate simultaneously the most likely genotypes and allele frequency in a population of N samples, emitting an accurate posterior probability of there being a segregating variant allele at each locus as well as for the genotype of each sample. It can make accurate SNP and INDEL calls on both single sample and multi-sample data.

The next step in data analysis is filtering and identifying putative causal variants. Assumptions and a rational method for removing unlikely causal variants are required due to the large numbers of variants known to be present in any individual (>3 million). Roughly 85-90% of these variants have already been reported and can be found in dbSNP. On average, ~10,000 SNPs are non-synonymous or stops, of which 1500 are not contained in dbSNP. There are ~ 30 million (M) variants catalogued in dbSNP (build 132), of which 20 M have been validated. To analyze our exome sequencing data, after consultation with Dr. Peter White, we excluded any exonic variants with <10 reads or <20% non-reference SNP. Those remaining variants with frequency >1% in 1000 Genomes Project or the NHLBI Exome Variant Project data were also excluded, leaving only rare known and novel variants.

Data are then arranged by family according to the pedigree structure (sporadic trio, multiplex family) and searched for de novo and inherited variants that meet the above criteria (≥10 reads, ≥20% non-reference SNP or in/del). Variants are further annotated using the proprietary version of the Human Gene Mutation Database (38), purchased by the BGC, which provides predictions of the functional effect of amino acid substitutions (SIFT, PolyPhen2, MutationTaster, Grantham), nucleotide sequence conservation scores (GERP, PhyloP), a comprehensive catalog of known disease-associated genes and mutations (OMIM, HGMD), and pathway information. "Probably damaging variants" receive the highest priority for further analysis, including searches against a candidate autism gene list that we have generated that encompasses published known or likely susceptibility loci.

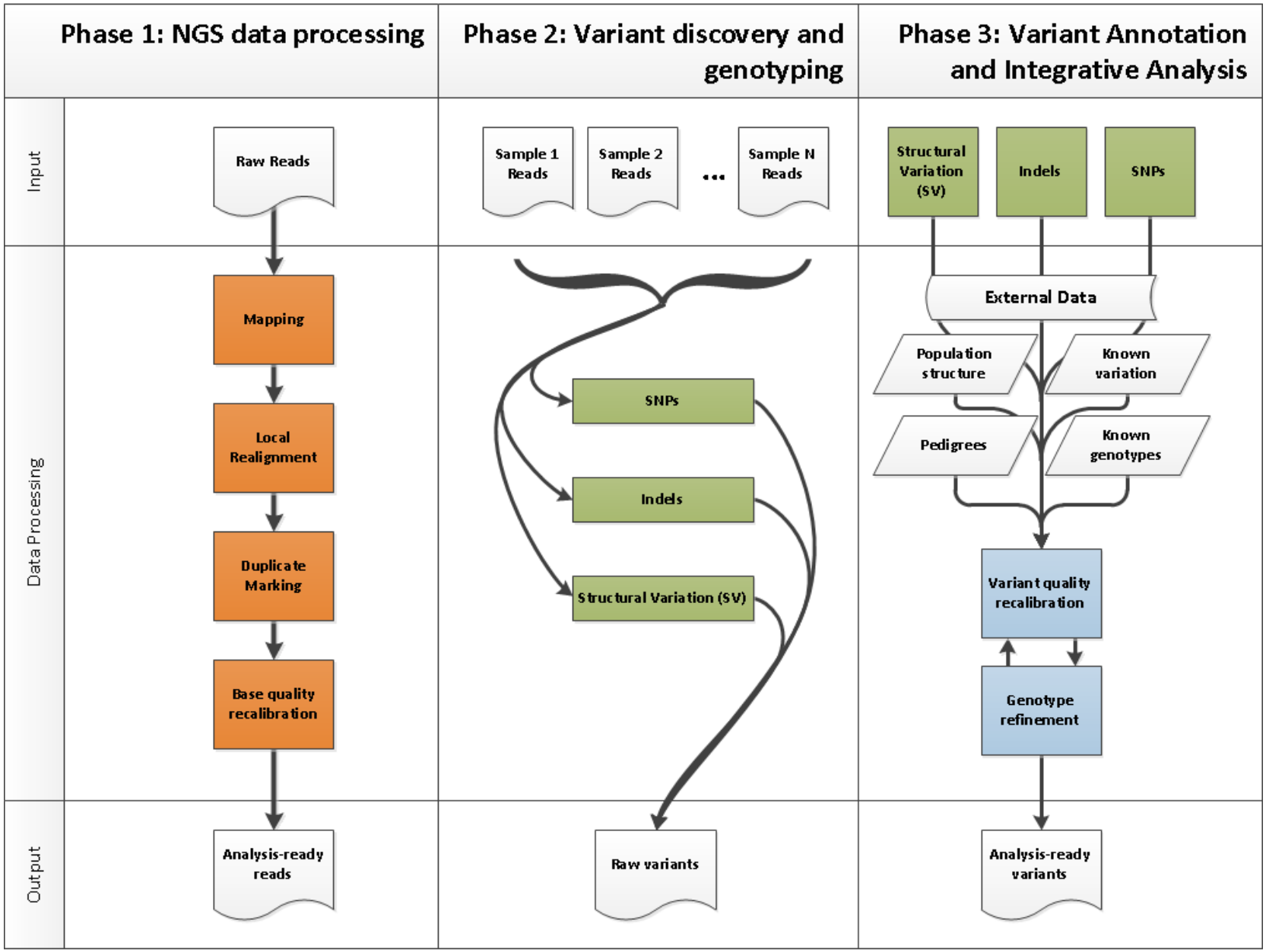


Figure 5. Biomedical Genomics Core Sequence Analysis

While analysis of the exome data from the families sequenced is on-going, we have completed verification of potential de novo damaging coding variants in the first 13 sporadic families (Table 17). Potentially damaging de novo variants were suspected in 7 of the 13 probands. Primers were designed to span the variant and confirmatory PCR performed using standard Sanger sequencing. All 15 of the variants that passed our criteria (≥10 reads, ≥20% non-reference SNP) were confirmed to be de novo. For those with lower confidence, 14 were not confirmed, and one variant was confirmed but inherited from a normal parent. For all of the probands, we were able to use primary DNA extracted from blood to confirm the de novo variant, excluding possible artifacts from cell line preparation (Table 18). Several of these genes are of interest as candidate ASD susceptibility loci. Further analyses of these variants, as well as analysis of the remaining sequenced samples will continue in the new grant.

Table 17. Sanger Sequencing Validation of De Novo Variants in First 13 Sporadic Trios

Individual	# Potential de novo Variants Validated	Confirmed de novo	Not Present	Inherited	Genes with Confirmed de novo variant
A87	0	0	0	0	
A168	4	4	0	0	BMI1; CADPS2; DIAPH3; MIDN
A211	2	0	2	0	
A311	3	0	3	0	
A379	2	1	1	0	DPYSL3
A439	4	2	2	0	TJP3; MUC16
A510	2	0	2	0	
A545	6	5	1	0	CACNA1H; KCNMB4; COL6A5; DYM; YLPM1
A645	1	1	0	0	AZIN1
A717	2	1	1	0	NREP
A735	0	0	0	0	
A744	1	0	1	0	
A754	3	1	1	1	SEMA4C

Table 18. Verified De Novo Variants from 13 Sporadic Families

Individual	CytoBand	GeneSymbol	Gene Name	Amino Acid Substitution	GERP[1]	SIFT[2]	PolyPhen2[3]
A379	5q32	DPYSL3	dihydropyrimidinase-like 3	S427Y	5.47	0.13	0.39
A439	19p13.3	TJP3	tight junction protein 3 (zona occludens 3)	F438V	4.5	0	0.79
A439	19p13.2	MUC16	mucin 16, cell surface associated	F10305S	-1.77	0.04	
A545	3q22.1	COL6A5	collagen, type VI, alpha 5	H2373Y	4.47		
A545	12q15	KCNMB4	potassium large conductance calcium-activated channel, subfamily M, β-member 4	K200N	1.64	0.13	0.84
A545	14q24.3	YLPM1	YLP motif containing 1	S657L	5.93	0	
A545	16p13.3	CACNA1H	calcium channel, voltage-dependent, T type, alpha 1H subunit	V869I	3.96	0.23	
A545	18q21.1	DYM	dymeclin	A43E	5.87	0.01	0.02
A645	8q22.3	AZIN1	antizyme inhibitor 1	C217Y	5.1	0.02	1
A717	5q22.1	NREP	neuronal regeneration related protein homolog (rat)	V36G	2.13	0	
A754	2q11.2	SEMA4C	semaphorin 4C	D305E	-4.16	1	0
A168	7q31.32	CADPS2	Ca++-dependent secretion activator 2	A1165T	5.47	0.01	
A168	10p12.2	BMI1	BMI1 polycomb ring finger oncogene	splicing variant	4.77		
A168	13q21.2	DIAPH3	diaphanous homolog 3 (Drosophila)	R37L	5.25	0	0.93
A168	19p13.3	MIDN	midnolin	C20G	2.62	0	0.99

[1]GERP provides a conservation score at nucleotide position ranging from -12.3 to 6.7; higher numbers indicate higher conservation

[2]SIFT predicts the effect of an amino acid substitution ranging from 0 to 1; scores less than 0.05 are predicted to affect protein structure

[3]PolyPhen2; predicts the effect of an amino acid substitution with scores ranging from 0 to 1; higher scores indicate more effect.

7.0 Aim 4 – Cost Analysis and Satisfaction Surveys

Dr. Seiber and his staff at OSU performed a business case analysis showing how the services provided in Aim 2 benefited Air Force beneficiaries and examined the cost of providing the services. This business case analysis included (1) satisfaction surveys of Air Force beneficiaries and (2) a cost effectiveness analysis of both the new multi-disciplinary assessment clinic and the new treatment services (individual psychological consultations and social skills groups), and of coordination of care.

To document the value of the project, the vendor conducted:

1. Satisfaction surveys with Air Force parents of participating children
2. Interviews with Air Force parents of participating children
3. Document review of appointment waiting times
4. Interviews with clinicians participating in the project
5. Document review of project expenditures

Satisfaction surveys with Air Force parents of participating children.
The sample population was comprised of Air Force members whose child or children participated in either the Comprehensive Autism Assessment Team (CAAT), a multidisciplinary assessment clinic for children suspected of having autism, or the Social Skills Group for Autistic Children at Dayton Children's Medical Center (DCMC). The participants were recruited over a period of 2 years from the clinic and social skills groups. The CAAT clinic sees roughly 2 children every other month, or approximately 6 times per year. Twelve social skills groups were offered, and each group included 6-8 children from military and civilian families. At the end of their services either in the CAAT clinic or the social skills group, military parents received a cover letter explaining the study and an optional survey to fill out and mail to the investigators anonymously. Two parent satisfaction surveys were created for (1) families receiving care through the CAAT clinic (Appendix B) and (2) families of children attending a social skills group for autistic children (Appendix C). These two groups were surveyed separately because they utilized different types of services and because they are two different populations. Children utilizing the clinic are expected to be younger and early in the diagnosis process. The social skills group members are young adolescents who have been diagnosed with autism as younger children. Aspects of satisfaction measured include access to necessary services, waiting time for diagnosis, availability of appointments and physician/clinic communication.

Satisfaction with CAAT clinic: Parents were highly satisfied with the CAAT clinic. They rated each of the health care providers (developmental pediatrician, psychologist and occupational therapist) quite highly, with an average of 9.5, 9.75 and 9 out of a possible 10, respectively. More detailed questions assessed parents' perception of the developmental pediatrician in 12 areas of patient care (Table 19; n=4), including skill in managing their child's condition, coordination of services, communication, answering questions and understanding how the child's

condition affects the family. Ratings were provided on a 5-point scale where 1=poor to 5=excellent. Parents rated their developmental pediatrician as "excellent" on all measures, except one that received a slightly lower score, at 'very good'. This measure was related to flexibility in working with the family.

Among those who responded to the clinic survey, all were white, nearly all of the affected children were male and none had other siblings with autism. Children were between 3-5 years old, with an average age of 3.75 years. Parents had seen at least 1 other doctor prior to coming to the clinic and consistently reported scheduling was "very easy."

Table 19. Average Rating by Parents of CAAT Clinic Users of Developmental Pediatricians

Dimension of care	Average Rating
This person's skill in managing your child's condition is…	5.00
This person's ability to provide general health care, like the care your child would need for a cold or the flu is…	5.00
When it comes to helping you coordinate services for your child, this person does a(n) ….. job.	5.00
When it comes to communicating with other professionals about your child's care, this person does a(n) ….. job.	5.00
This person's effort to be flexible in the way that he/she works with your family is…	4.68
This person's sensitivity to your family's cultural background and your beliefs about health is…	5.00
When it comes to really listening to your opinions about your child's care this person does a(n) ….. job.	5.00
This person's ability to answer your questions regarding your child's condition is…	5.00
The amount of information and guidance this person gives you to help prevent future problems for your child is…	5.00
When it comes to referring you to other doctors or services that your child needs, this person does a(n) ….. job.	5.00
This person's effort to put you in touch with other parents who have similar concerns is…	5.00
When it comes to understanding how your child's condition affects your family, this person has a(n) …. understanding	5.00

Satisfaction with social skills groups: Among parents whose children took part in the social skills groups, child ages ranged from 10-15 years, with an average of 12.6 years. Most were white and most were male, and none had siblings with autism. Parents rated the social skills class as "somewhat" or "very satisfied," with an average score of 4.15 out of 5. More than 90% would recommend the class to other parents of children with autism. Topics parents felt were most useful included conversation skills, learning to take turns, dealing with bullying and working in groups. Parents would have liked to have seen topics such as recognizing cues from others, dealing with siblings and other peer relationships, and overall more time for each topic. Only one parent expressed dissatisfaction with the class. This parent felt the emphasis on bullying was too strong and resulted in her child fixating on the idea of bullying. Most parents also recognized that each child with autism is very different which makes designing a group class a challenge. When asked what they would change about the class, most parents said they wished it were longer and offered on a more regular basis.

The survey also included items assessing the use and availability of a variety of autism-related services (Table 20; n=13). For each service, parents were asked if they had ever used the service, if they were currently using that service, and to rate the availability, quality and need for the service on a 5 point scale. For behavioral treatment services, most had used or were currently using this service. They rated availability (3.25 out of 5), greater quality (3.57) and even greater need (4.33). A similar and stronger trend was noted for social skills training.

Table 20. Parent Experiences and Perceptions with Autism-Related Services

	% Currently using/used previously	Availability (average rating, scale 1-5)	Quality (average rating, scale 1-5)	Need (average rating, scale 1-5)
Behavioral treatment	85%	3.25	3.57	4.33
Social Skills	100%	2.90	4.00	4.75
Speech-language therapy	92%	3.20	3.44	3.50
Occupational therapy	85%	3.85	4.00	4.20
Family Services	31%	2.00	2.50	4.00
Autism specialty clinics/centers	31%	2.25	4.50	4.67
Diagnostic services	70%	3.00	4.00	5.00

Interviews with Air Force parents of participating children

Parents who completed either the clinic or social skills survey were asked if they would be willing to participate in individual interviews regarding their experiences related to accessing autism care for their child. Any family willing to participate in an individual interview then provided contact information on a form separate from their survey instrument to maintain anonymity of the survey, and were contacted by the research team directly. A total of 6 parents agreed to be interviewed, 1 interview with a parent who had used the multidisciplinary clinic and 5 with parents who had used the social skills groups. These interviews gathered more detailed information than the survey about the process of seeking a diagnosis for their child. The interview questions are included in Appendix D.

For the parent whose child utilized the CAAT clinic, the diagnosis process was straightforward. The family first suspected their child might have autism when he was approximately 20 months old. At the child's 2 year checkup, the parents spoke with the base pediatrician who provided a referral to the CAAT clinic. Approximately 1 month later, the child was seen at the clinic, requiring only a 15 minute drive from the family's home, and the child was diagnosed as having autism. The child's mother reported that the process was very convenient, the setting in which her child was observed allowed him to be active, and she felt the various specialists involved provided her with adequate information.

Parents of children in the social skills group also described their process in seeking a diagnosis. These children were all older, ranging from 10-15 years old, and the CAAT clinic was not available at the time their children were diagnosed. The families had been dealing with autism for a much longer time, 6-8 years compared to 1 year for the clinic child. All parents interviewed had moved during the process of getting a diagnosis or accessing services. For these families, the time from initial suspicion to diagnosis ranged considerably. Two families reported approximately 1 year, while others reported up to 6 years to obtain an official diagnosis. Several reported that they would begin the diagnosis process with one pediatrician only to move and have to begin again in a new location. Often there is difficulty in accessing services in a new location because of long waiting times for appointments with specialists. One parent described her experience in this way:

"The main issue was the length of time between visits. Appointments were 2-3 months apart. The wait was very stressful as we were trying to figure out what our son needed. Also, finding qualified professionals for various testing who were covered by Tricare was challenging at times. You need a doctor who "already knows the paved way to get there" and I had to be very persistent in advocating for my child."

While parents in this group reported longer times to diagnosis, they also reported that once their child was diagnosed, they felt it was possible to get the necessary services for their child. They reported few difficulties after diagnosis other than those associated with relocation and finding new service providers. Parents of children in this group overwhelmingly supported the idea of a

multidisciplinary clinic and wished it had been available when their children were younger. They also stressed the need for education for parents and general pediatricians about services available, and the importance of early diagnosis.

Document review of appointment waiting times

DCMC provided the research team with data regarding the length of time between physician referral to DCMC and initial visit with the psychology assessment department. This data was limited to children who were referred for assessment related to autism, including those who utilized the CAAT clinic. Children who were inpatients or being seen in another clinic at the time of their contact with the psychologist for autism were excluded from this analysis. Waiting times were examined prior to the study and followed through completion for both military and non-military families (Table 21).

Table 21. Average Waiting Times from Referral to Psychologist Appointment

Grant status	Quarter	Days from referral to appointment		
		Military family	Non-military family	Overall
Pre-grant award	1/1/09-3/31/09	17.0	21.8	20.8
	4/1/09-6/30/09	20.4	28.3	26.8
	7/1/09-9/30/09	22.0	28.4	27.5
Post-grant award	10/1/09-12/31/09	31.3	29.4	29.9
	1/1/10-3/31/10	19.4	34.9	32.3
	4/1/10-6/30/10	21.1	27.7	25.8
	7/1/10-9/30/10	31.6	23.8	25.7
	10/1/10-12/31/10	33.0	23.3	26.8
	1/1/11-3/31/11	18.4	25.2	20.9
	4/1/11-6/30/11	41.5	33.3	35.3
	7/1/11-9/30/11	8.3	25.3	20.6
	10/1/11-12/31/11	21.1	24.4	22.9
	1/1/12-3/31/12	38.3	25.4	27.7
	4/1/12-6/30/12	26.1	23.7	24.8
	7/1/12-9/30/12	21.5	26.2	24.4

From January 2009 to October 2012, there were 668 autism-related referrals made to DCMC. Of these, 472 were outpatient or not being seen in another clinic at the time of referral and were thus included in the analysis. The majority (78%) of these were non-military families with only 22% coming from military families. During the course of the study, there was considerable variation in waiting times, and waiting times increased for both groups. Prior to initiation of this study in October 2009, military families waited an average of 19.20 days compared to 25.92 days for non-military families. Over the duration of the study period, the average waiting time for military families was 25.32 days compared to 27.53 for non-military families. This increase in waiting time for military families may be due to the fact that some of these referrals included children who were seen in the multidisciplinary clinic. Because the clinic was scheduled only every other month, children may have had to wait longer for a clinic appointment. However, once they were seen in the clinic, this was generally the only appointment needed. In addition, because military families comprised a significantly smaller portion of the sample, individuals with a longer waiting time can have a greater effect on the average waiting time than in the larger portion of non-military families. These waiting times compare favorably with those noted in other studies. In a study of 6 cities in Ohio, average median waiting time for a child psychiatric appointment was 62 days (39). Nationally, one study documented an average waiting time of 77 days (40). In this study, the average waiting time was less than one month, considerably shorter than Ohio and National averages.

Interviews with clinicians participating in the project

Interviews were conducted with the six providers who were most directly involved with the services provided through the grant. These were two physicians, a psychologist, a post-doctoral psychology fellow, a social worker, and an occupational therapist. These interviews gathered provider perspectives on the benefits enabled by the CAAT clinic, the education sessions and the social skills groups (Table 22).

CAAT clinic: Three benefits emerged for military families: convenience, comprehensiveness, and clarity. First, the CAAT clinic provides convenience to families in what providers called a “one stop” approach that avoids the prolonged waiting times of traditional referral approaches to diagnosis. The CAAT clinic approach condenses to one day the time required to go from initial examination to final diagnosis. For military families, this condensed timeline is particularly beneficial as it reduces the number of days the active duty parent must miss work and avoids any interruption of the diagnostic process that could occur if the family is relocated during the diagnostic process.

Second, providers said that, as a result of co-locating the physicians, psychologist, and therapists, the CAAT clinic provides families with a comprehensive answer: a complete evaluation, diagnosis, and set of recommendations. Families leave with all of their questions answered and information about not only the medical services available, but also the social and psychological services as well.

Third, by providing a more comprehensive understanding of the child's condition, the CAAT clinic approach gives families greater clarity as well. Providers said that as a result of working together, they could hear each other's conversations with families and build their own explanations on those of others. Furthermore, any ambiguities or seeming contradictions in the statements of different providers are readily apparent and easily addressed, allowing for better integration of the medical, psycho-social, and therapeutic information provided to families.

Social Skills Groups: Analysis of provider interviews revealed three themes in terms of benefits enabled by the educational sessions and social skills groups: access to valuable but otherwise unavailable services, behavioral improvements, and networking. First, these additional services filled an important gap: as the psychologist pointed out, there had been a demand for such services for a number of years, but no such services were available in the community. Not only did these services provide useful information and skills training, they also gave families access to professionals who could answer questions and provide advice for individual needs.

Second, providers pointed to noticeable behavioral improvements in children who attended the education sessions and skills groups as evidence of the benefits and effectiveness of these services. Providers reported observing children learn to make friendships, maintain eye contact, and pay attention, significant accomplishments for children with autism. In fact, as children learned new behaviors they modeled them for one another. At annual reunions, providers said parents would report that their children were still using many of the skills they had learned in the groups. One parent reported to the psychologist that her child's teacher, noting an improvement in the child's behavior, asked if the child was on new medication. The mother said, "no," crediting the social skills groups with the remarkable transformation.

Third, the education sessions and skills groups provided parents of children with autism a chance to network. They were able to meet and interact with others who were dealing with many of the same issues, to learn from one another's experiences, and to exchange contact information to help sustain ongoing relationships and mutual support.

Table 22. Summary of Benefits of CAAT Clinic and Social Skills Groups Perceived by Providers

Group	Benefit	Details
CAAT clinic	Convenience	• Condenses to one day the time required to go from initial examination to final diagnosis • Reduces the number of days the active duty parent must miss work • Avoids any interruption of the diagnostic process that could occur if the family is relocated during the diagnostic process.
	Comprehensiveness	• As a result of co-locating the physicians, psychologist, and therapists, the CAAT clinic provides families with a comprehensive answer: a complete evaluation, diagnosis, and set of recommendations
	Clarity	• Providers could hear each other's conversations with families and build their own explanations on those of others. • Any ambiguities or seeming contradictions in the statements of different providers are readily apparent and easily addressed, • Better integration of the medical, psycho-social, and therapeutic information provided to families
Social Skills group	Access to valuable but otherwise unavailable services	• Demand for such services for a number of years, but no such services available in the community. • Services provide useful information and skills training • Provide families with access to professionals who could answer questions and provide advice for individual needs
	Behavioral improvements	• Providers reported observing significant accomplishments for children with autism. • As children learned new behaviors they modeled them for one another
	Networking	• Education sessions and skills groups provided parents of children with autism a chance to network. • Meet and interact with others who were dealing with many of the same issues • Learn from one another's experiences • Exchange contact information to help sustain ongoing relationships and mutual support

Document review of project expenditures

Expenditures for Aim 2 totaled $557,000 for two years (subsequently expended over three years). These funds purchased (1) a reorganization of diagnostic care for military families with autistic children; (2) protected access to providers at DCMC; and (3) social skills and education groups for older children previously diagnosed with autism. To estimate costs for the separate activities, we focus on recurring costs (Table 23). These recurring costs provide the business case for what would be needed to continue the activity, after the higher, initial start-up expenditures. Non-recurring project costs are allocated to the reorganization of diagnostic care.

Reorganization of diagnostic care – The key element purchased by the project funds was a successful reorganization of diagnostic care for military families. Both providers at DCMC and WPAFB have committed to continuing the multidisciplinary clinic after the project ends. This reorganization of care indicates that military families can now obtain both resolution and clarity for suspected cases of autism within one month instead of the one to six years experienced by Air Force families who did not have access to the multidisciplinary clinic. This prompt resolution prevents duplicative care after relocation and provides clarity for military families who can subsequently obtain appropriate services for an autistic child. This reorganization, providing both higher quality of care and patient satisfaction, was obtained for less than $334,500, total cost. This total includes all fixed and non-recurring expenditures associated with the project, excluding the $95,000 per year required to continue the social skills and education groups and $16,250 for the protected access to providers at DCMC. Funding to continue the multidisciplinary CAAT clinic will be covered by fee-for-service billing by DCMC to TriCare and by ongoing provider salaries at WPAFB.

Protected access to providers at DCMC – Protected time from the lead psychologist at DCMC will not be continued after the project ends, but DCMC's commitment to continuing the multidisciplinary clinic ensures continued priority access to diagnostic services for WPAFB families. Reserving four hours per week of the lead psychologist's time for military families cost $16,250 per year.

Social skills and education groups – The social skills and education groups for older children with autism will not be continued after the project ends. Participating families expressed high satisfaction and enthusiasm for these groups and credited them with improved outcomes for their children. Continuation of these services requires funding to retain the social worker hired by the project at $60,000 per year and 0.5 FTE of the post-doctoral fellow at $35,000 per year (salary, fringes, plus indirect costs).

Table 23. Cost Summary of CAAT Clinic and Social Skills/Educational Groups

	Grant funds	Required to continue service
Reorganization of care	$334,500	$0/yr
Protected appointment time for military families	$32,500	$16,250/yr
Social Skills and Education groups	$190,000	$95,000/yr
Total	$557,000	$111,250/yr

8.0 Conclusions

Major accomplishments of the project are summarized here.

- Over 260 families were enrolled in CORA (32% from WPAFB).
- As part of CORA enrollment, research microarrays were performed on 86 affecteds (79**%** from WPAFB) who could not easily obtain a clinical array test.
    - Eight probands had pathogenic or likely pathogenic copy number variants (CNVs), including 2 families from WPAFB with large pathogenic unbalanced chromosome translocations.
- In 2010, WPAFB developmental pediatricians partnered with DCMC to develop a new comprehensive autism assessment team (CAAT) for military families. Since inception, thirteen CAAT clinics have been held, staffed by DCMC psychologists and WPAFB developmental pediatricians, with 23 children from military families assessed. A rapid, multidisciplinary approach is the preferred model of care for diagnosis of an ASD.
    - Upon review of the multidisciplinary clinic model, DCMC determined that the CAAT clinic could be self-sustaining. DCMC plans to continue offering the CAAT clinic and will make it available for military and civilian families.
- Utilizing SNaPshot technology, 6 SNPs in *CHRNA5* and SNPs in 4 additional genes (*CNTNAP2, SLC1A1, HTR5A,* and *HTR2A)* demonstrated significant allelic-expression imbalance (AEI). Deep sequencing of the transcriptome identified an additional 20 genes with significant AEI.
- Association studies were completed on up to 158 trios from our autism cohort examining 12 variants with AEI. One candidate gene (*HTR2A)* involved in serotonin signaling demonstrated a significant association (p=0.0003).
- Protocols were established for whole exome and transcriptome next-generation sequencing.
    - Whole exome sequencing has been completed on 39 families (132 individuals) and data analysis has begun.
    - Transcriptome sequencing has been completed by Dr. Sadee's lab on 5 brain tissue samples.
- Satisfaction surveys completed by parents and providers for the CAAT clinics and Social Skills groups revealed overall high levels of satisfaction with both services.

- Benefits of utilizing the CAAT clinic during the diagnostic process for military families included: convenience of a local, multidisciplinary, one-day clinic appointment; comprehensiveness – a complete evaluation, diagnosis, and recommendations; and clarity of the medical, psycho-social, and therapeutic information provided.

- Benefits of the Social Skills groups for military families included access to otherwise unavailable services, behavioral improvements in the children attending the groups, and networking opportunities for parents.

- The cost effectiveness analysis of the CAAT clinic revealed that the reorganization of the separate diagnostic components (medical, psychological, and treatment) into a multidisciplinary clinic resulted in prompt diagnosis, prevention of duplicative care, higher quality of care, and high levels of parental satisfaction.

    - The funding needed to continue the CAAT clinic can be covered by fee-for-service billing and is a self-sustaining model.

9.0 Publications, Abstracts, and Posters

Publications **All publications received DoD approval.*

Cottrell, C.E, Bir, N., Varga, E., Alvarez, C.E., Bouyain, S., Zernzach, R., Thrush, D.V, Evans, J., Trimarchi, M., Butter, E.M, Cunningham, D., Gastier-Foster, J.M, McBride, K.L., and Herman, G.E. Contactin 4 as an autism susceptibility locus. Autism Res. 2011; 4(3): 189-99.

Smith, R.M., Papp, A.C., Webb, A., Ruble, C.L., Munsie, L.M., Nisenbaum, L.K., Kleinman, J.E., Lipska, B.K., & Sadee, W. (in press). Multiple regulatory variants modulate expression of 5-hydroxytryptamine 2A receptors in human cortex. Biol Psychiatry. 2013; 73(6): 546-54.

Abstracts/Posters **All abstracts and posters received DoD approval.*

Dr. Randall Zernzach, Col, USA, MC gave an oral plenary presentation titled "*Contactin 4 as a Possible Autism Susceptibility Locus*" at the 2011 Military Health System Conference on 1/27/2011.

Ms. Emily Hansen and Mr. Wesley Banks presented a poster titled "*Analysis of Candidate Genes for ASDs within the Central Ohio Registry for Autism*" at the joint Nationwide Children's Hospital and The Ohio State University internal research retreat on 4/6/2011.

Dr. Ryan Smith, PhD from The Ohio State University presented a talk at the International Meeting for Autism Research (IMFAR) on 5/13/2011 titled "*Allelic mRNA Expression of Cellular Adhesion Molecules, Glutamate and GABAergic Genes, and RNA Splicing Modulators in Typically-Developed and ASD Frontopolar Cortex*."

10.0 References

1. MMWR. (2012) Prevalence of Autism Spectrum Disorders – Autism and Developmental Disabilities Monitoring Network, 14 Sites, United States, 2008. Centers for Disease Control and Prevention MMWR, 61(3):1-19.
2. Geschwind DH. (2011) Genetics of autism spectrum disorders. Trends Cogn Sci, 15(9):409-16.
3. Miller DT, Adam MP, Aradhya S, Biesecker LG, Brothman AR, Carter NP, Church DM, Crolla JA, Eichler EE, Epstein CJ and others. (2010) Consensus Statement: Chromosomal Microarray is a First-Tier Clinical Diagnostic Test for Individuals with Developmental Disabilities or Congenital Anomalies. The American Journal of Human Genetics, 86(5):749-764.
4. Riggs ER, Wain KE, Riethmaier D, Smith-Packard B, Faucett WA, Hoppman N, Thorland EC, Patel VC, Miller DT. (2013). Chromosomal microarray impacts clinical management. Clinical Genetics, cge.12107.
5. Iossifov I, Ronemus M, Levy D, Wang Z, Hakker I, Rosenbaum J, Yamrom B, Lee Y-H, Narzisi G, Leotta A and others (2012). De novo gene disruptions in children on the autistic spectrum. Neuron, 74(2):285-199.
6. Neale BM, Kou Y, Liu L, Ma/'ayan A, Samocha KE, Sabo A, Lin C-F, Stevens C, Wang L-S, Makarov V and others (2012). Patterns and rates of exonic de novo mutations in autism spectrum disorders. Nature, 485(7397):242-245.
7. O'Roak BJ, Vives L, Girirajan S, Krakoc E, Krumm N, Coe BP, Levy R, Ko A, Lee C, Smith JD and others (2012). Sporadic austim exomes reveal a highly interconnected protein network of de novo mutations. Nature, 485(7397):246-250.
8. Sanders SJ, Murtha MT, Gupta AR, Murdoch JD, Raubeson MJ, Willsey AJ, Ercan-Sencicek AG, DeLullo NM, Parikshak NN, Stein JL and others (2012). De novo mutations revealed by whole-exome sequencing are strongly associated with autism. Nature, 485(7397):237-241.
9. Maehama T, Dixon JE (1999). PTEN: A tumor suppressor that functions as a phospholipid phosphatase. Trends Cell Bio, 9:125-128.
10. Eng C (2003). PTEN: One gene, many syndromes. Hum Mutat, 22:183-198.
11. Hobert JA, Eng C (2009). PTEN hamartoma tumor syndrome: an overview. Genet Med, 11:687-694.
12. Goffin A, Hoefsloot LH, Bosgoed E, Swillen A, Fryns JP (2001). PTEN mutation in a family with Cowden syndrome and autism. Am J Med Genet, 105:521-524.
13. Parisi MA, Dinulos MB, Leppig KA, Sybert VP, Eng C, Hudgins L (2001). The spectrum and evolution of phenotypic findings in PTEN mutation positive cases of Bannayan-Riley-Ruvalcaba syndrome. J Med Genet, 38:52-58.
14. Zori RT, Marsh DJ, Graham GE, Marliss EB, Eng C (1998). Germline PTEN mutation ina family with Cowden syndrome and Bannayan-Riley-Ruvalcaba syndrome. Am J Med Genet, 80:399-402.

15. Butler MG, Dasouki MJ, Zhou XP, Talebizadeh Z, Brown M, Takahashi TN, Miles JH, Wang CH, Stratton R, Pilarski R, Eng C (2005). Subset of individuals with autism spectrum disorders and extreme macrocephaly associated with germline PTEN tumour suppressor gene mutations. J Med Genet, 42:318-321.
16. Buxbaum JD, Cai G, Chaste P, Nygren G, Goldsmith J, Reichert J, Anckarsater H, Rastam M, Smith CJ, Silverman JM, Hollander E, Leboyer M, Gillberg C, Verloes A, Betancur C (2007). Mutation screening of the PTEN gene in patients with autism spectrum disorders and macrocephaly. Am J Med Genet B Neuropsychiatr Genet, 144B:484-491.
17. Herman GE, Butter E, Enrile B, Pastore M, Prior TW, Sommer A (2007). Increasing knowledge of PTEN germline mutations: two additional patiens with autism and macrocephaly. Am J Med Genet A, 143:589-593.
18. Varga EA, Pastore M, Prior T, Herman GE, McBride KL (2009). The prevalence of PTEN mutations in a clinical pediatric cohort with autism spectrum disorders, developmental delay, and macrocephaly. Genet Med, 11:111-117.
19. McBride KL, Varga EA, Pastore MT, Prior TW, Manickam K, Atkin JF, Herman GE (2010). Confirmation study of PTEN mutations among individuals with autism or developmental delays/mental retardation and macrocephaly. Autism Res, 3:137-141.
20. Lim JE, Papp A, Pinsonneault J, Sadee W, Saffen D (2006). Allelic expression of serotonin transporter (SERT) mRNA in human pons: lack of correlation with the polymorphism SERTLPR. Mol Psychiatry, 11:649-662.
21. Pinsonneault JK, Papp AC, Sadee W (2006). Allelic mRNA expression of X-linked monoamine oxidase a (MAOA) in human brain: dissection of epigenetic and genetic factors. Hum Mol Genet, 15:2636-2649.
22. Lim JE, Pinsonneault J, Sadee W, Saffen D (2007). Tryptophan hydroxylase 2 (TPH2) haplotypes predict levels of TPH2 mRNA expression in human pons. Mol Psychiatry,12:491-501.
23. Johnson AD, Zhang Y, Papp AC, Pinsonneault JK, Lim JE, Saffen D, et al (2008). Polymorphisms affecting gene transcription and mRNA processing in pharmacogenetic candidate genes: detection through allelic expression imbalance in human target tissues. Pharmacogenet Genomics, 18:781-791.
24. Smith RM, Alachkar H, Papp AC, Wang D, Mash DC, Wang JC, Bierut LJ, and Sadee W (2011). Nicotinic α5 receptor subunit mRNA expression is associated with distant 5' upstream polymorphisms. Eur J Hum Genet, 19:76-83.
25. Abrahams BS, Geschwind DH (2008). Advances in autism genetics: on the threshold of a new neurobiology. Nat Rev Genet, 9:341-355.
26. Alarcon M, Abrahams BS, Stone JL, Duvall JA, Perederiy JV, Bomar JM, et al (2008). Linkage, association, and gene-expression analyses identify CNTNAP2 as an autism-susceptibility gene. Am J Hum Genet, 82:150-9.

27. Handley MT, Lian LY, Haynes LP, and Burgoyne RD (2010). Structural and functional deficits in a neuronal calcium sensor-1 mutant identified in a case of autistic spectrum disorder. PLoS One, 5:e10534.
28. Kumar RA, Sudi J, Babatz TD, Brune CW, Oswald D, Yen M, Nowak NJ, Cook EH, Christian SL, and Dobyns WB (2010). A de novo 1p34.2 microdeletion identifies the synaptic vesicle gene RIMS3 as a novel candidate for autism. J Med Genet, 47:81-90.
29. Smith RM, Papp AC, Webb A, Ruble CL, Munsie LM, Nisenbaum LK, Kleinman JE, Lipska BK, and Sadee W (in press). Multiple regulatory variants modulate expression of 5-hydroxytryptamine 2A receptors in human cortex. Biol Psychiatry. doi: 10.1016/j.biopsych.2012.09.028
30. Stefansson H, Ophoff RA, Steinberg S, Andreassen OA, Cichon S, Rujescu D, et al. (2009) Common variants conferring risk of schizophrenia. Nature, 460:744-747.
31. Cottrell CE, Bir N, Varga E, Alvarez CE, Bouyain S, Zernzach R, et al. (2011) Contactin 4 as an autism susceptibility locus. Autism Res. 4:189-99.
32. O'Roak BJ, Deriziotis P, Lee C, Vives L, Schwartz JJ, Girirajan S, et al. Exome sequencing in sporadic autism spectrum disorders identifies severe de novo mutations. Nat Genet. 2011;43:585-9.
33. Neale BM, Kou Y, Liu L, Ma'ayan A, Samocha KE, Sabo A, et al. Patterns and rates of exonic de novo mutations in autism spectrum disorders. Nature. 2012;485:242-5.
34. Chahrour MH, Yu TW, Lim ET, Ataman B, Coulter ME, Hill RS, et al. Whole-exome sequencing and homozygosity analysis implicate depolarization-regulated neuronal genes in autism. PLoS Genet. 2012;8:e1002635.
35. Iossifov I, Ronemus M, Levy D, Wang Z, Hakker I, Rosenbaum J, et al. De novo gene disruptions in children on the autistic spectrum. Neuron. 2012;74:285-99.
36. Sanders SJ, Murtha MT, Gupta AR, Murdoch JD, Raubeson MJ, Willsey AJ, et al. De novo mutations revealed by whole-exome sequencing are strongly associated with autism. Nature. 2012;485:237-41.
37. DePristo MA, Banks E, Poplin R, Garimella KV, Maguire JR, Hartl C, et al. A framework for variation discovery and genotyping using next-generation DNA sequencing data. Nat Genet. 2011;43:491-8.
38. Stenson PD, Mort M, Ball EV, Howells K, Phillips AD, Thomas NS, et al. The Human Gene Mutation Database: 2008 update. Genome Med. 2009;1:13.
39. Steinman KJ, Kelleher K, Dembe AE, Wickizer TM, Hemming T., (2012) The use of a "mystery shopper" methodology to evaluate children's access to psychiatric services, *J Behav Health Serv Res*, Jul;39(3):305-13.
40. Bisgaier J, Levinson D, Cutts DB, & Rhodes KV., (2011) Access to autism evaluation appointments with developmental-behavioral and neurodevelopmental subspecialists, *Arch Pediatr Adolesc Med*, Jul;165(7):673-4.

APPENDIX A

Social Skills Group Topics and Handouts

Week	Session Topic	Materials*
1	Introduction: Group purpose & rules, binders provided, Knowledge of Social Skills pre-test (KSS); get-to-know-you game (information trading); How to be a friend (Part I): friendliness, social reciprocity	CFT, SST, HtbF, NSW, AAB, Y&M
2	Show & Share; How to be a friend (Part II): How *not* to be a friend & respectful interactions/getting along with others, flexibility, avoiding Rule Police behavior	HtbF, SST, SS, AAB, OEMP, FIHF-1, SBB-Change
3	Nonverbal communication – Be a social skills detective: *think with your eyes*, gestures & facial expressions; good listening position	CFT, SST, SSPB, WTL, NSW, SS, TS, AAB, MMF/P
4	Talking & listening nicely (I): *Using your H.E.A.D.* (voice tone, eye gaze, taking turns, and maintaining personal space); sensitive topics	CFT, SST, NSW, SSPB, PR, SOS, AAB, OEMP, FIHF-1
5	Talking & listening nicely (II): Starting, maintaining and ending two-way conversations - topic maintenance, 'Wh' questions, Two-Question rule	CFT, SST, SS, SSPB, NSW, Y&M, SOS, FIHF-1, MMP
6	Playing Nicely/Play Dates (I): Choosing friends, sportsmanship, joining play, taking 'no' for an answer; Teasing [vs. criticism] (I); (**HW**: play date #1)	AAB, SS, CFT, SSPB, OEMP, HTBF, SBB-Sharing, MMB/F
7	Playing Nicely/Play Dates (II): Being a good host/guest; Teasing (II) - joking vs. mean teasing, tools for handling teasing and bullying; (**HW**: play date #2)	SST, CFT, SSPB, BPB, SBN, FIHF-2, MFRW, MMB/P
8	KSS post-test; Review; Graduation celebration: games, food, certificates; (Parent feedback meetings)	

Books and Manuals (*****with Materials Abbreviation Key):

CFT – *Children's Friendship Training* (2003), by Fred Frankel, Ph.D. and Robert Myatt, Ph.D.

SST – *Social Skills Training for Children and Adolescents with Asperger's Syndrome and Social- Communication Problems* (2003), by Jed Baker, Ph.D.

SS – *Super Skills: A Social Skills Group Program for Children with Asperger Syndrome, High Functioning Autism and Related Disorders* (2005), by Judith Coucouvanis

NSW – *Navigating the Social World: A Curriculum for Individuals with Asperger's Syndrome, High Functioning Autism and Related Disorders* (2002), by Jeanette McAfee, M.D.

SOS – *S.O.S. – Social Skills in Our Schools* (2006), by Michelle A. Dunn

SSPB – *The Social Skills Picture Book* (2003), by Jed Baker, Ph.D.

TS – *Think Social* (2005), by Michelle Garcia Winner

HtbF – *How to be a Friend: A Guide to Making Friends and Keeping Them* (*Dino Tales: Life Guides for Families*; 1998), by Laura Krasny Brown and Marc Brown

WTL – *What's That Look on Your Face? All About Faces and Feelings* (2008), by Catherine S. Snodgrass

OEMP – *Oops! Excuse Me Please! And Other Mannerly Tales* (1998), by Bob McGrath

PR – *Playing It Right: Social Skills Activities for Parents and Teachers of Young Children with Autism Spectrum Disorders, Including Asperger Syndrome and Autism* (2006), by Rachael Bareket

AAB – *All About Boundaries: Teaching Children About "Drawing the Line"* (2008), by Tonia Caselman, Ph.D., L.C.S.W. and Beth Cohen, M.S.W., L.C.S.W.

Y&M – *The You and Me Workbook: A Book That Teaches Social Skills and Social Awareness* (2001), by Lisa M. Schab

BPB – *Bullies are a Pain in the Brain (1997)*, by Trevor Romain

DVDs:

FIHF-1 - *Fitting In and Having Fun – Social Skills Training Video Series, Volume 1*

SBB - *Skill-Building Buddies: Sharing and Taking Turns* (2008); *Handling Transitions and Change* (2007)

MM[B,F,P] - *Model Me Confidence and Bullying Prevention* (2009), *Model Me Friendship* (2007), *Model Me Time for a Playdate* (2005)

SBN- *Stop Bullying Now Campaign Video Toolkit* (2006; http://takeastand.stopbullying.gov/kids/webisodes

APPENDIX B

CAAT Multidisciplinary Clinic Parent Satisfaction Survey

Wright-Patterson/Dayton Children's Hospital

Multidisciplinary Autism Assessment Clinic

Parent Survey

Survey number: _____

Information about your child:

Child's current age:_____

Age at which you were first concerned about autism:_____

Child's Race: _____

Child's gender:_____

Number of siblings: _____ (number of siblings with autism):_____

Thoughts about the Multidisciplinary Clinic

Overall, how would you rate the care you receive from the following specialists at the clinic:

Specialist	Rating
Developmental Pediatrician	1 2 3 4 5 6 7 8 9 10
Occupational Therapist	1 2 3 4 5 6 7 8 9 10
Psychologist	1 2 3 4 5 6 7 8 9 10

Thinking about the developmental pediatrician your child sees at the clinic, how would you rate:

	Excellent	Very Good	Good	Fair	Poor	Does Not Apply
1. This person's skill in managing your child's condition is…						
2. This person's ability to provide general health care, like the care your child would need for a cold or the flu is…						
3. When it comes to helping you coordinate services for your child, this person does a(n) ….. job.						
4. When it comes to communicating with other professionals about your child's care, this person does a(n) ….. job.						
5. This person's effort to be flexible in the way that he/she works with your family is…						
6. This person's sensitivity to your family's cultural background and your beliefs about health is…						
7. When it comes to really listening to your opinions about your child's care this person does a(n) ….. job.						
8. This person's ability to answer your questions regarding your child's condition is…						
9. The amount of information and guidance this person gives you to help prevent future problems for your child is…						
10. When it comes to referring you to other doctors or services that your child needs, this person does a(n) ….. job.						
11. This person's effort to put you in touch with other parents who have similar concerns is…						
12. When it comes to understanding how your child's condition affects your family, this person has a(n) …. understanding						

Questions about your experience with autism-related care for your child:

1. How many doctors did you see before coming to the Multidisciplinary Clinic?

_____

2. How difficult was it to make an appointment at other facilities?

Very Difficult	Somewhat Difficult	Neither Difficult nor Easy	Somewhat Easy	Very Easy

3. How difficult was it to make an appointment at the Multidisciplinary Clinic?

Very Difficult	Somewhat Difficult	Neither Difficult nor Easy	Somewhat Easy	Very Easy

4. What was the most useful about the Multidisciplinary Clinic?

_____

_____

_____

5. What would you change about the Multidisciplinary Clinic?

_____

_____

_____

6. Would you recommend the Multidisciplinary Clinic to a friend with an autistic child?

_____

_____

_____

APPENDIX C

Social Skills Group Parent Satisfaction Survey

Wright-Patterson/Dayton Children's Hospital

Social Skills Group

Parent Survey

Survey number: _____

Information about your child:

Child's current age:_____

Age at which you were first concerned about autism:_____

Race: _____

Child's gender:_____

Number of siblings: _____ (number of siblings with autism):_____

Questions about participation in the social skills group:

1. How satisfied are you the social skills group?

Very Satisfied	Somewhat Satisfied	Neither Satisfied nor Unsatisfied	Somewhat Unsatisfied	Very Unsatisfied

2. What topics or activities in the group were most useful for your family?

_____

_____

_____

3. What topics or activities do you wish had been included in the group?

_____

_____

_____

4. Would you recommend this social skills group to a friend with an autistic child?

   Yes No

5. Is there anything you would change about the social skills group?

   _____

   _____

   _____

Questions about your previous experience with autism-related care for your child:

Please tell us your experience with the following services:

	Using currently	Used in past	Availability	Quality	need
Behavioral treatment			1 2 3 4 5	1 2 3 4 5	1 2 3 4 5
Social Skills training			1 2 3 4 5	1 2 3 4 5	1 2 3 4 5
Speech-language therapy			1 2 3 4 5	1 2 3 4 5	1 2 3 4 5
Occupational therapy			1 2 3 4 5	1 2 3 4 5	1 2 3 4 5
Family services			1 2 3 4 5	1 2 3 4 5	1 2 3 4 5
Autism specialty clinics/centers			1 2 3 4 5	1 2 3 4 5	1 2 3 4 5
Diagnostic Services			1 2 3 4 5	1 2 3 4 5	1 2 3 4 5

APPENDIX D

Qualitative Interview Questions

Parent Satisfaction

1. How many children with autism do you have?
2. How long has your child (or children) had autism?
3. When did you first suspect autism?
4. What type of doctor did you first consult about your concerns?
5. Tell me about your initial experiences leading to an autism diagnosis.
    a. How long did it take to schedule an initial assessment?
    b. Did you encounter any process-related difficulties in seeking a diagnosis?
    c. Did you or anyone in your family have to take time off of work to take your child to appointments to receive a diagnosis? If so, how frequently?
    d. How much travel time do you think was involved?
6. If you used the Multidisciplinary Clinic at Dayton Children's Medical Center:
    a. What did you like about your experience there?
    b. Was there anything that was difficult about the process?
    c. What do you think the Air Force or Dayton Children's Medical Center could do differently?
7. If you used the Social Skills groups at Dayton Children's Medical Center:
    a. What did you like about your experience there?
    b. Was there anything that was difficult about the process?
    c. What do you think the Air Force or Dayton Children's Medical Center could do differently?
8. Is there anything else you'd like to add about your experiences in seeking a diagnosis for your child?

APPENDIX E

Qualitative Interview Questions

Provider Interview Questions

[Prompt for specific stories]

1. Tell me about your role in the CAAT clinic and/or social skills groups or educational sessions.

2. Tell me about a typical day for you in the CAAT clinic and/or social skills groups or educational sessions?

3. From your perspective, what are the biggest benefits or advantages?

    a. (prompt) for families/children?
    b. (prompt) for you and other providers?

4. How has the CAAT clinic and/or social skills groups or educational sessions impacted your work?

5. If you didn't have the CAAT clinic and/or social skills groups or educational sessions, what would be different? What could you not do?

6. How would you improve the CAAT clinic and/or social skills groups or educational sessions?

ACRONYMS AND ABBREVIATIONS

ADI-R	Autism Diagnostic Interview – Revised
ADHD	Attention Deficit Hyperactivity Disorder
ADOS	Autism Diagnostic Observation Schedule
AEI	Allelic Expression Imbalance
AF	Air Force
ASD	Autism Spectrum Disorder
BGC	Biomedical Genomics Core
BRRS	Bannayan-Riley-Ruvalcaba syndrome
BWA	Burrows-Wheeler Aligner
CAAT	Comprehensive Autism Assessment Team
CASAVA	Consensus Assessment of Sequence and Variation
cDNA	complementary DNA
CMA	Chromosomal microarray
CNS	central nervous system
CNV	copy number variation
CORA	Central Ohio Registry for Autism
CS	Cowden syndrome
DCMC	Dayton Children's Medical Center
DD	Developmental Delay
DNA	deoxyribonucleic acid
DoD	Department of Defense
EFMP	Exceptional Family Member Program
FISH	Fluorescence in situ hybridization
FTE	full-time employee
GATK	Genome Analysis Toolkit
GERP	Genomic Evolutionary Rate Profiling
GWAS	genome-wide association studies
HGMD	Human Gene Mutation Data
INDEL	Insertion/Deletion
IQ	Intelligence Quotient
IRB	Institutional Review Board
MR	Mental Retardation
NCH	Nationwide Children's Hospital
NGS	Next Generation Sequencing
NHLBI	National Heart, Lung, and Blood Institute
NIH	National Institute of Health
OMIM	Online Mendian Inheritance in Man
OSU	Ohio State University
PCR	Polymerase Chain Reaction
qPCR	Qualitative Polymerase Chain Reaction
PI	Principal Investigator
PFC	Prefrontal Cortex
PhyloP	Phylogenetic P-Values
PLS	Proteus-like syndrome
PolyPhen2	Polymorphism Phenotyping v2
PTEN	Phosphatase and tensin homologue deleted on chromosome ten
RNA	Ribonucleic acid
SIFT	Scale-Invariant Feature Transform
rSNP	regulatory polymorphism
SNP	single nucleotide polymorphism
SNV	Single nucleotide variants
UTR	Untranslated Retion
VCF	Variant Call Files
VNTR	Variable Nucleotide Tandom Repeat
VUS	Variant of Unknown Significance
WPAFB	Wright-Patterson Air Force Base